United States Patent [19]

Friedman, Jr. et al.

[11] **Patent Number: 4,975,109**

[45] **Date of Patent: Dec. 4, 1990**

[54] **MICROBIOCIDAL COMBINATIONS OF MATERIALS AND THEIR USE**

[75] Inventors: **Lester A. Friedman, Jr.; Richard F. McFarlin**, both of Atlanta, Ga.

[73] Assignee: **Lester Technologies Corp.**, Atlanta, Ga.

[21] Appl. No.: **189,379**

[22] Filed: **May 2, 1988**

[51] **Int. Cl.**[5] **A01N 31/00**; A01N 33/00; D21N 21/04; D21N 21/36

[52] **U.S. Cl.** **71/67**; 162/161

[58] **Field of Search** 71/67; 162/161

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,374,754	1/1945	Kreidl et al.	167/14
2,768,949	2/1956	Hewey	210/6
3,248,281	2/1966	Goodenough	167/17
3,252,855	5/1966	Wehner et al.	167/22
3,306,810	2/1967	Buckman et al.	162/161
3,377,275	4/1968	Michalski et al.	210/64
3,393,058	2/1968	Oppermann	44/68
3,426,134	7/1969	Shema et al.	424/302
3,524,812	3/1970	Shema et al.	210/63
3,574,697	4/1971	Welcher	260/454
3,647,703	3/1972	Shema et al.	252/180
3,674,457	2/1972	Wolfson et al.	71/67
3,690,860	9/1972	Salutsky et al	71/67
3,817,828	4/1974	Bendiner	162/161
3,860,713	8/1974	Shema et al.	424/426
3,864,053	3/1975	Shema et al.	210/62
3,864,253	12/1975	Shema et al.	210/62
3,879,559	11/1975	Shema et al.	424/270
3,896,229	11/1975	Shema et al.	424/270
3,896,231	6/1975	Shema et al.	424/304
3,897,554	6/1975	Shema et al.	424/248
3,897,562	6/1975	Shema et al.	424/304
3,908,030	11/1975	Shema et al.	424/270
3,928,198	12/1975	Brink, Jr. et al.	210/62
3,929,561	12/1975	Shema et al.	162/161
3,929,562	12/1975	Shema et al.	162/161
3,929,563	12/1975	Shema et al.	162/161
3,930,015	12/1975	Swered et al.	424/298
3,947,581	1/1976	Swered et al.	424/270
3,994,772	3/1976	Swered et al.	162/161
4,010,277	3/1977	Swered et al.	424/298
4,200,633	4/1980	Quinlan	424/246
4,200,634	4/1980	Quinlan	424/246
4,285,765	2/1981	Pera et al.	162/161
4,295,932	10/1981	Pocius	162/161
4,411,799	8/1983	Ito et al.	210/753
4,451,376	4/1984	Sharp	210/701
4,518,610	6/1985	Umekawa et al.	514/516
4,539,071	3/1985	Clifford et al.	162/161
4,579,665	6/1986	Davis et al.	210/755
4,594,091	6/1986	Girvan	71/67
4,595,691	3/1986	LaMarre et al.	514/367
4,597,940	2/1986	Hager	422/32
4,644,021	2/1987	Toda et al.	523/122
4,648,978	4/1987	Makinen et al.	210/759
4,655,936	4/1987	Stuebner	210/764
4,661,503	4/1987	Martin et al.	514/372
4,661,517	7/1987	Martin et al.	514/515
4,661,518	8/1987	LaMarre et al.	514/528
4,684,469	7/1987	Pedersen et al.	210/632
4,720,492	1/1988	Quinlan	514/222

OTHER PUBLICATIONS

Du Pont Industrial Chemicals Department Product Data Sheet, "Oxone", Monopersulfate Compound (1985).

Index Guide, Chem. Abst., 1981, p. 1347G, 11th Coll. Index., Chem. Abst., p. 47874GS.

Baldry, Chem. Abst., vol. 102 (1985), 218211B.

Primary Examiner—Richard L. Raymond

Assistant Examiner—Brian M. Burn

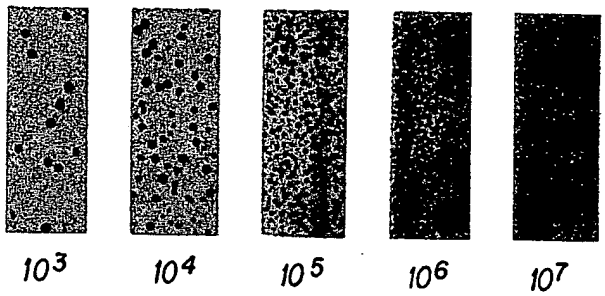
Attorney, Agent, or Firm—Birch, Stewart, Kolasch & Birch

[57] **ABSTRACT**

The present invention is directed to microbiocidal combinations and processes for inhibiting the growth of microorganisms. The novel combinations and processes of the present invention show unexpected activity against microorganisms, including bacteria, fungi and algae. Specifically, the combinations of materials which are to be added to a system at the time of their use comprise (i) an oxidant such as potassium monopersulfate, sodium perborate, hydrogen peroxide or sodium percarbonate, (ii) a microbiocide such as 2,2-dibromo-3-nitrilopropionamide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one, tetrahydro-3,5-dimethyl-2H,1,3,5-thiadiazine-2-thione, and sodium dimethyldithiocarbamate/disodium ethylene bis dithiocarbamate and optionally (iii) a surfactant such as a fluorinated surfactant, and (iv) an anti-corrosive material such as an anhydrous phosphate, or combinations thereof.

29 Claims, 2 Drawing Sheets

FIG. 1



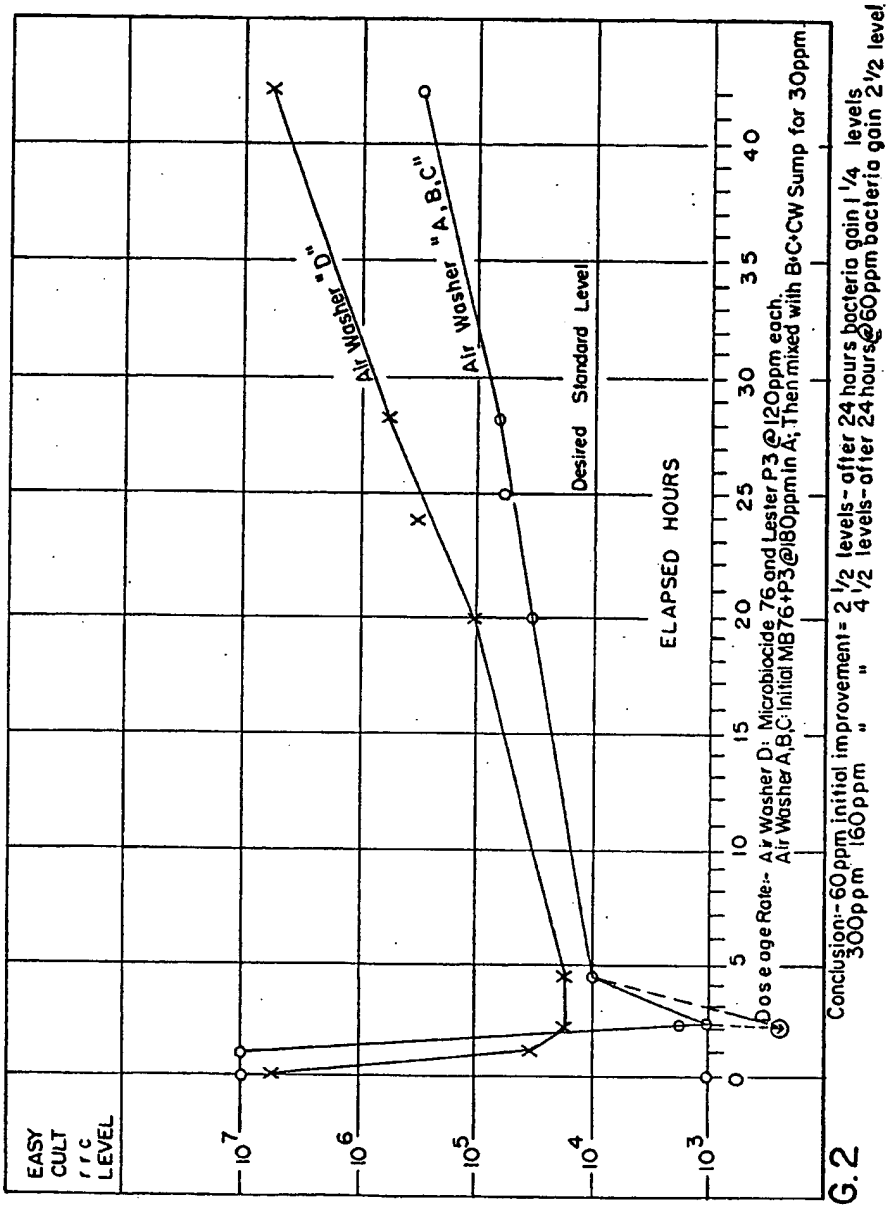

EASY CULT r r c LEVEL
10^7
10^6
10^5
10^4
10^3
0
5
10
15
20
25
30
35
40
ELAPSED HOURS
Desired Standard Level
Air Washer "D"
Air Washer "A,B,C"
Dosage Rate:- Air Washer D: Microbiocide 76 and Lester P3 @ 120ppm each.
Air Washer A,B,C: Initial MB76+P3@180ppm in A; Then mixed with B+CW Sump for 30ppm
Conclusion:- 60ppm initial improvement = 2 1/2 levels - after 24 hours bacteria gain 1/4 levels
300ppm 160ppm " " 4 1/2 levels - after 24 hours @ 60ppm bacteria gain 2 1/2 level

FIG. 2

MICROBIOCIDAL COMBINATIONS OF MATERIALS AND THEIR USE

FIELD OF THE INVENTION

The present invention relates to microbiocidal combinations of materials and processes utilizing these combinations for inhibiting the growth of microorganisms. More particularly, the combinations of materials which are to be added to a system at the time of their use comprise (i) an oxidant such as potassium monopersulfate, sodium perborate, hydrogen peroxide or sodium percarbonate, (ii) a microbiocide such as 2,2-dibromo-3-nitrilopropionamide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl -4-isothiazolin-3-one, or tetrahydro-3,5-dimethyl-2H,1,3,5-thiadiazine-2-thione, sodium dimethyldithiocarbamate/disodium ethylene bisdithiocarbamate and optionally (iii) a surfactant such as a fluorinated surfactant, sodium and (iv) an anti-corrosive material such as an anhydrous phosphate, or combinations thereof.

BACKGROUND OF THE INVENTION

The formation of slime by microorganisms is a problem which commonly occurs in many systems. For example, slime commonly forms in cooling water systems, lagoons, lakes, ponds, pulp and paper mill systems, petroleum operations and in industrial lubricants and coolants. In cooling systems which employ large amounts of water as the cooling medium, the formation of slime by microorganisms is a significant and constant problem. Problematic microorganisms include bacteria, sulfate reducing bacteria, fungi and algae which produce slime in aqueous systems where such slime is objectionable from either an operational or an aesthetic point of view.

Moreover, airborne microorganisms are readily entrained in water from cooling towers because this medium is an ideal growth environment for microbiocidal growth. Various types of microorganisms flourish in the cooling tower itself while other organisms grow in such areas as the piping, the tower sump and in the passages of the cooling system. Typically, the slime acts to deteriorate towers made of wood or it promotes corrosion when deposited on metal surfaces of cooling systems. Furthermore, the slime tends to plug or foul pipes and valves and to form deposits within heat exchange surfaces thereby reducing heat exchange or cooling efficiency.

Pulp and paper mill systems also operate at conditions which encourage the growth of microorganisms resulting in similar fouling problems discussed hereinabove. Moreover, microorganisms become entrained in the paper product itself causing breakage on the paper machines which necessitates the shutting down of the paper making process. As a result, production time is lost because the equipment must be cleaned and the value of the slime containing product is reduced because of its poor quality.

Slime may also be objectionable from the standpoint of cleanliness and sanitation in breweries, wineries, dairies and other industrial plants or establishments. Moreover, sulfate reducing bacteria may become problematical in waters used for the secondary recovery of petroleum or for oil drilling in general. For example, these organisms are able to reduce sulfates present in the injection water to sulfides which in turn react with soluble iron salts to form insoluble iron sulfide. Matted

deposits composed of sulfides, occluded oil and other solids are thereby produced which is undesirable since water containing such deposits when injected underground may plug subterranean formations. In addition, sulfate reducing bacteria cause the corrosion of metal by accelerating galvanic action. Accordingly, microbiological corrosion is a well recognized problem in the petroleum industry.

Moreover, ponds, lakes, lagoons or pools used either for industrial purposes or for pleasure often become suitable environments for microbial growth, especially during warm weather. Health problems including infection may result from the growth of microorganisms in recreational areas. In addition, microorganisms cause further problems during industrial storage and these organisms must be eliminated, for example, prior to use of the stored materials.

In addition, lubricants and cutting fluids are prepared by mixing organic compounds with water to produce solids, emulsions or suspensions in many metal forming operations. Bacteriological contamination of these fluids is inevitable due to the heat and dirt found in many metal working plants. These fluids must be discarded when biological contamination is too severe.

Accordingly, because of the problems discussed hereinabove in various industrial processes, numerous biocidal materials have been recommended in order to eliminate or to reduce microbial growth. Various materials have enjoyed widespread use in such applications including chlorine, chlorine dioxide, organo-mercurials, chlorinated phenols, organo-bromines and various organo-sulfur compounds. However, each of these generally useful materials is deficient for a variety of reasons. For instance, chlorination is limited by its specific toxicity for slime-forming organisms at economic levels by the pH of the aqueous medium and by the ability of chlorine to react before its full biocidal function is achieved.

Moreover, economy is a significant consideration with regard to the use of known biocides. The cost of the biocide and the expense of its applications are examples of the economic factors which must be considered. Typically, the effectiveness of the known biocides is rapidly reduced as a result of exposure to physical conditions such as temperature or a reaction with ingredients in the system which results in a loss of biocidal effectiveness. Therefore multiple doses or large quantities of expensive biocidal chemicals have heretofore been required in order to maintain control over microbial growth.

It is, therefore, a principal object of the present invention to provide microbiocidal combinations of materials which are to be added to a system at the time of their use for controlling the growth of microorganisms.

It is another object of this invention to provide an improved process for controlling microorganisms in aqueous systems such as pulp and paper mill systems, cooling water systems, and petroleum operations.

These and other objects of the novel microbiocidal combinations of materials and processes of using the same of this invention will become apparent and are further described hereinbelow.

SUMMARY OF THE INVENTION

The present invention is directed to combinations of materials which are added to a system at the time of their use and which are used to control or inhibit micro-

bial growth. Specifically, the combinations of the present invention comprise (i) a microbiocidal effective amount of an oxidant such as potassium monopersulfate, sodium perborate, hydrogen peroxide or sodium percarbonate, (ii) a microbiocidal effective amount of a microbiocide such as 2,2-dibromo-3-nitrilopropionamide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-ene, or tetrahydro-3,5-dimethyl-2H,1,3,5-thiadiazine-2-thione, sodium dimethyldithiocarbamate/dissodium and optionally (iii) a surfactant such as a fluorinated surfactant, and (iv) an anti-corrosive material such as an anhydrous phosphate, or combinations thereof.

Furthermore, the combinations of the invention are utilized for controlling the growth and reproduction of microorganisms by adding an effective amount of the combination sufficient to control microbial growth in the system which is treated. The types of systems which are treated to control microbial growth include, but is not limited to, cooling water systems, pulp and paper mill systems, petroleum operations, industrial lubricants and coolants, lagoons, lakes, ponds, etc.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the EASI-CULT TTC model chart for visually estimating the number of cultures (e.g. bacteria/ml), and

FIG. 2 is a graph of EASI-CULT TTC level versus Time in hours.

DETAILED DESCRIPTION OF THE INVENTION

The foregoing objects are obtained by utilizing combinations of materials which are added to a system at the time of their use and which comprise mixtures of (i) oxidizing agents such as potassium monopersulfate, sodium perborate, hydrogen peroxide or sodium percarbonate, (ii) microbiocides such as 2,2-dibromo-3-nitrilopropionamide (DNP), methylene bis thiocyanate (MBT), 5-chloro-2-methyl-4 -isothiazolin -3-one/2 -methyl-4-isothiazolin-3-one (CMI) or, tetrahydro-3,5-dimethyl -2H,1,3,5-thiadiazine-2-thione, sodium dimethyldithiocarbamate disodium ethylene bis dithiocarbamate (TDD) and optionally (iii) a surfactant such as a fluorinated surfactant, and (iv) an anti-corrosive material such as anhydrous phosphate or combinations thereof. The resulting mixtures unexpectantly have a higher activity than that of the individual components which make up the mixtures.

According to the invention, a system is treated to inhibit the growth of the microorganisms with at least one microbiocide and at least one oxidant. These ingredients are typically added separately as individual compositions or components or they may be added to the system either concurrently or sequentially. The microbiocide is not combined with the oxidant well in advance of being added to the system because these materials adversely react over time when they are brought into direct contact with each other in concentrated form.

The combinations of this invention are utilized for controlling microbrial growth and reproduction in cooling water systems, pulp and paper mill systems, petroleum operations (e.g. oil well applications), industrial lubricants and coolants, lagoons, lakes and ponds, etc. The particular type of microorganisms present in these areas vary from location to location, and even at a given location over a period of time. Representative examples of microorganisms including fungi and bacteria which may be present at a given location and which need to be controlled include such genera as Asperoillus, Penicillium, Candida, Saccharomyces, Aerobacter, Escherichia, Alcaligenes, Bacillus, Chlorella, Spirogyra, Oscillatoria, Vaucheria, Pseudomonas, Salmonella, Staphylococcus, Pallularia, Flavobacterium and Rhizopus. The amount of the active ingredients of the invention which are to be added to these systems should be sufficient to control the microorganisms which are present in the system.

The amount of the microbiocides, the oxidant and optionally the surfactant and/or anti-corrosive material may be varied broadly while maintaining good biological control of such systems as cooling tower systems or paper making systems. For example, the preferable amount of 2,2-dibromo-3-nitrilopropronamide (DNP) in the system may be from about 2.5 ppm to about 10 ppm. In addition, the amount of potassium monopersulfate may be from about 5 ppm to about 60 ppm. However, in general, the amount of microbiocide and oxidant in the system may be between 2.5 ppm and 30 ppm. In addition, an equal amount of oxidant may be combined with the microbiocides (e.g. a 50:50 mix). Moreover, the amount of MBT in the system may be from about 10 ppm to about 45 ppm. When the microbiocides and oxidants are present in the above amounts, the resulting combination possesses a higher degree of effectiveness against microorganisms than the individual components comprising the mixture.

These resulting mixtures possess a high degree of slimicidal activity which could not have been predicted beforehand from the known activity of the individual ingredients comprising the mixture. Accordingly, it is therefore possible to produce a more effective slime-control agent than has previously been available. Because of the enhanced activity of the mixture, the total quantity of the biocide required for an effective treatment may be reduced.

Furthermore, approximately 0.4 grams to approximately 100 grams (about 400 ppm) of surfactant such as a fluorinated surfactant may be optionally added to the system. Suitable fluorinated surfactants include those manufactured by 3M such as FC-99, FC-100 and FC-129. FC-99 is an anionic surfactant which is a 25% active solution of amine-perfluoroalkyl sulfonates in water. FC-100 is an amphoteric surfactant which is a 28% active solution of fluorosurfactant solids in glycol/water. FC-129 is an anionic surfactant which is a 50% solution of potassium fluorinated alkyl carboxylates in water, butyl cellosolve and ethanol. Moreover, surfactants such as the alkylaryl polyether alcohols, polyether alcohols, alkyl benzene sulfonates and sulfates, and the like, may also be employed to enhance the dispersibility and stability of these formulations. In addition, sodium linear dodecyl benzene sulfonate may be a suitable surfactant. Also, a suitable class of surfactants is 0.5–50 ppm of propylene-oxide-ethylene oxide block copolymers which comprises a polyoxy-propylene glycol polymer having a molecular weight of from 1500–2000 which has been reacted with from 5–30% by weight of ethylene oxide. These latter compounds are primarily made by BASF as the Pluronic and Tetronic series. The fluorinated surfactants have been found to be about as effective as the pluronic series.

The invention further envisions the use of an anti-corrosive material as an optional ingredient. For instance, an anhydrous phosphate such as tetrapotassium pyro-

phosphate may be added to help protect mild steel from corrosive attack by the oxidant (e.g.monopersulfate). The anti-corrosive material may be blended with an oxidant such as OXONE before being added to the system. The anti-corrosive material may be present in an amount of from ½–50% based on the total amount of oxidant and anti-corrosive material in the mixture. More preferably, the amount of the anti-corrosive material is at least 1% of the total amount of oxidant and anti-corrosive material in the mixture. A typical formulation comprises 2% tetrapotassium pyrophosphate and 98% OXONE in order to produce the maximum amount of oxidizing power. Both sodium tripolyphosphate and tetrapotassium pyrophosphate are effective in reducing mild steel corrosion.

As to the amount of the combinations of materials to be added to the various systems, suitable and preferred quantities vary according to the specific system in which the combinations are used. As described above, when added to aqueous systems to control microorganisms, the suitable quantities vary from about 2.5 to 125 ppm of microbiocide to 1 to 125 ppm of oxidizing agent. Larger quantities of the microbiocides or oxidant may be used with no detrimental effect, but such large quantities increase the cost of treatment while having little additional benefit.

The microbiocides used in this invention are commercially available compounds or are easily synthesized from commercially available raw materials. Several representative microbiocides used in the invention and their suppliers are listed below in order of decreasing effectiveness.

The microbiocide, the oxidant and the optional materials may be added to the system sequentially or simultaneously. Moreover, the microbiocide may be metered into the system. In contrast, the solid oxidants, such as OXONE, may be added to the system by hand. Because some users prefer an all liquid system, it is possible to dissolve the solid oxidant in water and to add it to the system as a liquid.

2,2-dibromo-3-nitrilopropionamide (DNP) is a commercially available microbiocide (e.g. LESTER BAC-20), manufactured by Lester Laboratories, Inc., Atlanta, Georgia, or by the Dow Chemical Company of Midland, Michigan, (e.g. Dow XD-7287L).

Methylene bis thiocyanate (MBT), $CH_2(SCN)_2$, may be prepared for example, by the known procedure of reacting methylene bromide or iodide with an alkali metal or ammonium thiocyanate. MBT (e.g. MICROBIOCIDE 10) is a commercially available microbiocide manufactured by Lester Laboratories, Inc.

5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazol in-3-one (CMI) is a commercially available microbiocide manufactured by Lester Laboratories (e.g. MICROBIOCIDE-76) or by Rohm and Hass (e.g. KATHON 886).

Tetrahydro-3,5-dimethyl-2H,1,3,5-thiadiazine-2-thione (TDD) is a known compound and is commercially available from Lester Laboratories (e.g. MICROBIOCIDE 24) or from Stauffer Chemical (e.g. N-521).

Sodium dimethyldithiocarbamate/sodium ethylene bis dithiocarbamate (SDT) is a known formulation which is commercially available from Lester Laboratories (e.g., LESTER 30) or from VININGS Chemical (e.g., AMA-230).

Examples of microbiocides which did not respond to and were antagonistic to the oxidants tested were quaternary amine compounds such as the alkyl dimethylbenzyl amonium chloride family and (WSCP) poly[oxyethylene (dimethyliminio) ethylene (dimethyliminio) ethylene dichloride].

The oxidants used in the compositions of this invention are also commercially available compounds or are easily synthesized from commercially available raw materials. Several representative oxidizing agents useful in the invention are listed below.

Potassium monopersulfate is the most preferred oxidizing agent and is a known compound which is commercially available from DuPont as OXONE.

Additional oxidizing agents which are used in accordance with the present invention are sodium perborate, hydrogen peroxide and sodium percarbonate. Although the abovementioned oxidizing agents are preferred, a number of other oxidizing agents including potassium permanganate, sodium or ammonium persulfate and diperoxydodecanedoic acid may be used.

Suitable microbiocides and/or oxidants of this invention may be used diluted with a suitable solid or liquid carrier. Dusts may be prepared with a finely divided solid including talc, clay, pyrophyllite, diatomaceous earth, hydrated silica, calcium silicate, or magnesium carbonate. Moreover, wetting and/or dispersing agents may optionally be used. A wettable powder results when the proportions of these materials are increased which may be dispersed in water and applied from a spray.

Dusts may typically contain 1 to 15 percent of the microbiocides of this invention, while wettable powders may contain up to 50 percent or more of these compounds.

A typical formulation of a wettable powder comprises 20 percent to 50 percent of the suitable compounds of this invention, 45 percent to 75 percent of one or more finely divided solids, one percent to five percent of a wetting agent, and one percent to five percent of a dispersing agent. Typical wetting agents include sodium dodecyl sulfate, sodium nonylbenzene sulfonate, sodium dioctyl sulfosuccinate, octylphenoxypolyethoxyethanol, or other nonionic agents, such as ethylene and/or propylene oxide condensates with long chained alcohols, mercaptans, amines, or carboxylic acids. Typical dispersing agents include the sodium sulfonate of condensed napthalene-formaldehyde and lignin sulfonates.

Liquid concentrates may also be used. These are prepared by taking up the microbiocides of this invention in an organic solvent together with one or more surface active agents.

The microbiocides used in the present invention may be used in conjunction with other microbicidal agents and also in conjunction with miticides or insecticides or other pesticides.

The present invention is now described in greater detail by reference to the following examples which are given here for illustrative purposes only, and are not intended to limit the scope of the invention.

EXAMPLES

Test Procedure 1: Laboratory Tests

EASICULT-TTC test strips were used to evaluate the "kill power" of the various microbiocidal systems which were tested. EASICULT-TTC test strips are commercially available (Medical Technology Corp., Somerset, N.J.) and are culture paddles which are

dipped into a solution containing various microorganisms. The strips which have been dipped are closed within a container or vial and they are then incubated for 24 to 48 hours in an oven kept at a temperature range of 30°–40° C. The number of cultures (e.g. bacteria/ml) can be visually estimated from the EASICULT TTC model chart as shown in FIG. 1.

More specifically, for each test one four ounce sample jar and one EASICULT-TTC test strip is used. The test samples comprise about 1 ml to 100 ml of the solution to be tested. For example, about 100 ml of a sample is dosed at 30 ppm of a microbiocide such as MBT. If 1 ml were to have such a dose, it would mean that the original solution was 100 times as concentrated (e.g. 3000 ppm). This is equivalent to adding 3 grams of microbiocide to 1 liter of water. The proper amount of microbiocide is then added to the sample jar (e.g. 1 ml in the above example). The sample jar is then topped off with 100 grams of 1–10% of a suitable sample to be tested and is allowed to sit for 45 minutes in contact with the microbiocide/oxidant treatment, if any. After 45 minutes, an EASICULT-TTC test strip (e.g. paddle) is dipped into the prepared microbe/microbiocide solution and stirred for five complete circles in the jar. The paddle is withdrawn and tapped five times on the side of the jar to remove excess moisture. The paddle is replaced into its container which is then closed. The EASICULT-TTC unit is then incubated for 24–48 hours. At the end of this period the test strip is removed from incubation and is evaluated in the manner described by the EASICULT-TTC manufacturer. This procedure allows the number of cultures to be conveniently determined.

The microbial samples to be tested may be prepared as follows. For example, 1 gram of cooling tower slat scrapings is obtained from a cooling tower. In a 1 gram jug add 5 grams fructose and 5 grams of dehydrated culture broth to the scrapings. The jug is then topped off with deionized water. The sample to be tested is then allowed to grow for at least one week before use.

Test Procedure 2: Field Tests

The procedure for conducting field tests is to sample the actual test site (e.g. an air washer sump) with an EASICULT-TTC test strip 30 minutes before any chemicals are added. The microbiocide (e.g. CMI) is added to the test site (e.g. air washer sump) in a prespecified amount (e.g. 125 ppm). A prespecified amount of oxidant (e.g. OXONE) and optionally anti-corrosive material (e.g. phosphate) is then added. Preferably, an equivalent amount of P-3 (Oxone and phosphate) is used. EASICULT-TTC test strips are then used to sample the test site for a predetermined period (e.g. every half hour for four hours after adding the chemicals). Then, the test site is tested after longer intervals, for example, after eight hour periods.

EXAMPLE 1

Microbiocide tetrahydro-3,5-dimethyl-2H,1,3,5-thiadiazine-2-thione (MICROBIOCIDE 24) was evaluated using Test Procedure 1 to determine the effect when combined with microbiocide Poly(oxyethylene(dimethylimino)-ethylene-(dimethylimino) -ethylene dichloride) (e.g. ALGAECIDE 15) or with the oxidizing agent sodium perborate, or combinations thereof. The sample tested was taken from a waste water treatment plant of a specialty metal fabricating plant located in Carrollton, Georgia. The effectiveness of the various combinations is shown below in Table I.

TABLE I

Combinations of MicroBiocides and Oxidants Against Bacteria

Treatment	Ratio (a/b)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Control	0/0	0	10^4
a: MICROBIOCIDE 24/ b: Sodium Perborate	13/87	75/500	0
a: MICROBIOCIDE 24/ b: ALGAECIDE 15	43/57	75/100	10^2–10^3
a: MICROBIOCIDE 24/ b: —	100/0	75/0	10^3

The results show the improved effectiveness of MICROBIOCIDE 24 when used in combination with sodium perborate as the oxidizing agent. The oxidizing agent was more helpful than another known biocide.

EXAMPLE 2

Microbiocide 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl -4-isothiazolin-3-one (MICROBIOCIDE 76) was evaluated using Test Procedure 1 to determine the effect when combined with the oxidizing agent potassium mo (OXONE). The effect of combining SURFACTANT AEROSOL MA-80 with the oxidizing agent potassium monopersulfate (OXONE) was also evaluated. The sample tested was taken from a waste water treatment plant of a specialty metal fabricating plant in Carrollton, Georgia. The effectiveness of the various combinations is shown below in Table II.

TABLE II

Combinations of Microbiocides and Oxidants Against Bacteria

Treatment	Ratio (a/b)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
a: MICROBIOCIDE 76 b: —	100/0	20/0	10^5
a: MICROBIOCIDE 76/ b: OXONE	50/50	20/20	10^2
a: AEROSOL MA-80/ b: OXONE	50/50	20/20	10^4

The results show that the oxidizing agent potassium monopersulfate (OXONE) combined with the microbiocide was more effective than when combined with the surfactant (dispersant).

EXAMPLE 3

Microbiocides 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (MICROBIOCIDE 76) and methylene bisthiocyanate (MICROBIOCIDE 10) were evaluated using Test Procedure 1 to determine the effect when combined with surfactant AEROSOL

OT-75, oxidizing agent potassium monopersulfate (OXONE), or with a combination thereof. The sample tested was taken from a waste water treatment plant of a specialty metal fabricating plant in Carrollton, Georgia. The effectiveness of the various combinations are shown below in Table III.

TABLE III

Combinations of Microbiocides and Oxidants Against Bacteria			
Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Control	0/0/0	0/0/0	10^4
a: MICROBIOCIDE 76 b: — c: —	100/0/0	60/0/0	10^3
a: MICROBIOCIDE 76 b: AEROSOL OT-75 c: —	50/50/0	60/60/0	10^3
a: MICROBIOCIDE 76 b: — c: OXONE	50/0/50	60/0/60	0
a: MICROBIOCIDE 76 b: AEROSOL OT-75 c: OXONE	33/33/33	60/60/60	0
a: MICROBIOCIDE 10 b: — c: —	100/0/0	60/0/0	10^4
a: MICROBIOCIDE 10 b: AEROSOL OT-75 c: —	50/50/0	60/60/0	10^3-10^4
a: MICROBIOCIDE 10 b: — c: OXONE	50/0/50	60/0/60	0
a: MICROBIOCIDE 10 b: AEROSOL OT-75 c: OXONE	33/33/33	60/60/60	0

The results show the improved effectiveness of microbiocides MICROBIOCIDE 76 and MICROBIOCIDE 10 when used in combination with oxidizing agent potassium monopersulfate (OXONE) and optionally a surfactant (AEROSOL OT-75). Whereas the impact of the surfactant was marginal, the impact of the oxidizing agent (OXONE) combined with the microbiocides was dramatically more effective.

EXAMPLE 4

The effectiveness of the microbiocide methylene bis thiocyanate (MICROBIOCIDE 10) was evaluated using Test Procedure 1 to further evaluate the effect when combined with oxidizing agent potassium monopersulfate (OXONE) and optionally with surfactant (AEROSOL OT-75). The sample tested was taken from an air washer in a textile mill located in Talladega, Alabama. The effectiveness of the various combinations are shown below in Table IV.

TABLE IV

Combinations of Microbiocides and Oxidants Against Bacteria			
Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Control	0/0/0	0	10^5
a: MICROBIOCIDE 10 b: OXONE c: —	50/50/0	30/30/0	0
a: MICROBIOCIDE 10 b: OXONE c: AEROSOL-OT-75	33/33/33	30/30/30	0
a: MICROBIOCIDE 10 b: OXONE c: —	50/50/0	45/45/0	0

TABLE IV-continued

Combinations of Microbiocides and Oxidants Against Bacteria			
Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
a: MICROBIOCIDE 10 b: OXONE c: —	50/50/0	15/15/0	10^1

The results show that the use of the oxidant allows complete kill of the organisms at a fraction of the normal dosage level (30 ppm).

EXAMPLE 5

Microbiocide methylene bis thiocyanate (MICROBIOCIDE 10) was further evaluated using Test Procedure 1 to determine the extent of the effect when combined with oxidizing agent potassium monopersulfate (OXONE) and optionally with AEROSOL-OT-75. The sample tested was taken from an air washer in a textile mill located in Talladega, Alabama. The effectiveness of the various combinations are shown below in Table V.

TABLE V

Combinations of Microbiocides and Oxidants Against Bacteria			
Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results
Control	0/0/0	0	10^5
a: MICROBIOCIDE 10 b: OXONE c: —	50/50/0	5/5/0	10^5
a: MICROBIOCIDE 10 b: OXONE c: AEROSOL-OT-75	33/33/33	10/10/10	10^1
a: MICROBIOCIDE 10 b: OXONE c: AEROSOL-OT-75	33/33/33	15/15/15	0

The results show the improved effectiveness of MICROBIOCIDE 10 when used in combination with oxidizing agent potassium monopersulfate (OXONE) and optionally with AEROSOL OT-75.

EXAMPLE 6

Microbiocide methylene bis thiocyanate (MICROBIOCIDE 10) was again evaluated using Test Procedure 1 to determine the extent of the effect when combined with oxidizing agent potassium monopersulfate (OXONE). However, in these tests, a more potent or virulent selection of microorganisms was utilized. The sample tested was taken from an air washer in a textile mill located in Mission Valley, Texas. The effectiveness of the various combinations are shown below in Table VI.

TABLE VI

Combinations of Microbiocides and Oxidants Against Bacteria			
Treatment	Ratio (a/b)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Control	0/0	0	10^7
a: MICROBIOCIDE 10 b: OXONE	33/67	30/60	10^4
a: MICROBIOCIDE 10 b: OXONE	40/60	30/45	10^4
a: MICROBIOCIDE 10 b: OXONE	50/50	30/30	10^5
a: MICROBIOCIDE 10	67/33	30/15	10^7

TABLE VI-continued

Treatment	Ratio (a/b)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Combinations of Microbiocides and Oxidants Against Bacteria			
b: OXONE			
a: MICROBIOCIDE 10	75/25	30/10	10^7
b: OXONE			

The above results show effective concentrations of the various MICROBIOCIDE/OXONE combinations on the specific samples tested.

EXAMPLE 7

The effectiveness of microbiocide methylene bis thiocyanate (MICROBIOCIDE 10) was further evaluated using Test Procedure 1 to determine the extent of the effect when combined with oxidizing agent potassium monopersulfate (OXONE). However, in these tests the microorganisms were obtained from the textile mill in Talladega, Alabama. The effectiveness of the various combinations are shown below in Table VII.

TABLE VII

Treatment	Ratio (a/b)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Combinations of Microbiocides and Oxidants Against Bacteria			
Control	0/0	0	10^6
a: MICROBIOCIDE 10	50/50	30/30	0
b: OXONE			

EXAMPLE VIII

Microbiocides methylene bis thiocyanate (MICROBIOCIDE 10) and 2,2-dibromo-3-nitrilopropionamide (LESTER BAC-20) were evaluated using Test Procedure I to determine the effect when combined with oxidizing agents hydrogen peroxide (e.g. 8% H_2O_2), potassium monopersulfate (OXONE) and with the surfactant ZONYL FSJ (DuPont). The samples tested were taken from an air washer in effectiveness of the various combinations are shown below in Table VIII.

TABLE VIII

Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Combinations of Microbiocides and Oxidants Against Bacteria			
Control	0/0/0	0	10^6
a: MICROBIOCIDE 10	100/0/0	10/0/0	10^4
b: —			
c: —			
a: MICROBIOCIDE 10	91/9/0	10/1/0	10^4
b: 8% H_2O_2			
c: —			
a: MICROBIOCIDE 10	66/33/0	10/5/0	10^3
b: 8% H_2O_2			
c: —			
a: MICROBIOCIDE 10	50/25/25	10/5/5	10^3
b: 8% H_2O_2			
c: ZOYNL FSJ			
a: MICROBIOCIDE 10	50/50/0	10/10/0	10^3
b: 8% H_2O_2			
c: —			
a: BAC-20	66/33/0	10/5/0	10^1
b: 8% H_2O_2			
c: —			
a: BAC-20	50/50/0	10/10/0	0
b: 8% H_2O_2			

TABLE VIII-continued

Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Combinations of Microbiocides and Oxidants Against Bacteria			
c: —			
a: MICROBIOCIDE 10	40/60/0	10/15/0	10^3
b: OXONE			
c: —			
a: BAC-20	40/60/0	10/15/0	0
b: OXONE			
c: —			

These results show the improved effectiveness of microbiocides MICROBIOCIDE 10 and LESTER BAC-20 when used in combination with oxidizing agent hydrogen peroxide (e.g. 8% H_2O_2) or potassium monopersulfate (OXONE). Moreover, these results demonstrate that the combination of LESTER BAC-20 with each of the oxidizing agents is more effective than the respective combinations with MICROBIOCIDE 10. In other words, the combination of materials utilizing LESTER BAC-20 appears to be superior to the combination of materials using MICROBIOCIDE 10.

EXAMPLE IX

Microbiocides 5-chloro-2-methyl-4-isothiazolin -3-one/2-methyl-4-isothiazolin-3-one (MICROBIOCIDE 76), methylene bis thiocyanate (MICROBIOCIDE 10), and 2,2-dibromo-3 -nitrilopropionamide (LESTER BAC-20) were evaluated using Test Procedure 1 to determine the effect when combined with oxidizing agent hydrogen peroxide (e.g. 8% H_2O_2) and optionally the surfactant ZONYL. The test samples were taken from an air washer in a textile mill located in Mission Valley, Texas. The effectiveness of the various combinations are shown below in Table X.

TABLE X

Treatment	Ratio (a/b/c)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Combinations of Microbiocides and Oxidants Against Bacteria			
Control	0/0/0	0	10^4
a: MICROBIOCIDE 76	60/20/20	30/10/10	10^4
b: ZONYL			
c: 8% H_2O_2			
a: MICROBIOCIDE 76	33/33/33	10/10/10	10^5
b: ZONYL			
c: 8% H_2O_2			
a: MICROBIOCIDE 10	50/0/50	10/0/10	10^4
b: —			
c: 8% H_2O_2			
a: BAC-20	33/0/66	5/0/10	10^2
b: —			
c: 8% H_2O_2			

These results demonstrate that the combination of LESTER BAC-20 with 8% H_2O_2 is superior to the combination of the other materials tested with 8% H_2O_2.

EXAMPLE XI

Microbiocide 5-chloro-2-methyl-4-isothiazolin-3-one-2/ methyl-4-isothiazolin-3-one (MICROBIOCIDE 76) was further evaluated using Test Procedure 1 to determine the effect when it was combined with oxidizing agents potassium monopersulfate (OXONE), hydrogen peroxide (e.g. 6% H_2O_2), sodium perborate, potassium permanganate or sodium percarbonate or with MICROBIOCIDE 80. The samples tested were taken from

a cooling tower at Lester Laboratories in East Point, Georgia. The effectiveness of the various combinations are shown below in Table XI.

TABLE XI

Combinations of Microbiocides and Oxidants Against Bacteria

Treatment	Ratio (a/b)	Treatment Level (ppm)	EASICULT Test Results (bacteria/ml)
Control	0/0	0	10^7
a: MICROBIOCIDE 76 b: —	100/0	30/0	10^7
a: MICROBIOCIDE 76 b: OXONE	50/50	30/30	10^3
a: MICROBIOCIDE 76 b: 6% H_2O_2	50/50	30/30	10^6
a: MICROBIOCIDE 76 b: Sodium Perborate	50/50	30/30	10^6
a: MICROBIOCIDE 76 b: MICROBIOCIDE 80	50/50	30/30	0
a: MICROBIOCIDE 76 b: KBr-Sodium Perborate	50/50	30/30	10^5
a: MICROBIOCIDE 76 b: Potassium Permanganate	50/50	30/30	0
a: MICROBIOCIDE 76 b: Sodium Percarbonate	50/50	30/30	10^5

These results demonstrate the relative effectiveness of the various oxidizing agents when compared in use with a constant quantity of the microbiocide (MB-76). The combination of MICROBIOCIDE 76 with MICROBIOCIDE 80, a chlorinating agent, and potassium permanganate were effective for killing completely the bacteria. However, many of these alternate oxidants have intrinsic disadvantages including color, insoluble reaction products and corrosive tendencies.

EXAMPLE XII

A field test was conducted in accordance with Test Procedure 2 at the West Point Pepperell Mission Valley Mill in Texas. An air washer sump was sampled with an EASICULT-TTC test strip 30 minutes before any chemicals were added. MICROBIOCIDE 76 was added to the air washer sump to 125 ppm, followed by an equivalent amount of P-3(OXONE and phosphate). EASICULT-TTC strips were then dipped each half hour for four hours after the chemicals were added. Then, an interval of eight hours passed before another EASICULT-TTC test strip was used, followed by another eight hour interval before dipping another test strip. The initial EASICULT-TTC reading was 10^7. After about 2.53 hours the reading was reduced to 10^3. The results are plotted in FIG. 2.

EXAMPLE XIII

In order to establish the effectiveness of the combination in combatting or controlling slime formation which is experienced in various paper and pulp mills, the combinations of the invention are tested in the apparatus of a paper mill. Actual water samples are taken from pulp and paper mill systems which experience slime problems due to the microorganism population of the water. These slime problems are generally caused by a combination of microorganisms, which although primarily bacteria and fungi, in some cases also includes algae. As would be expected, the inventive combinations are added to the cooling water or the pulp and paper mill systems at any convenient point. The combinations are added upstream from the point or points at which microorganism control is desired in once-through or non-circulating systems. In circulating systems or pulp and paper systems, the combinations are added at any point, provided the time lapse and the conditions experienced between the point of addition and the point at which the effect of the combinations are experienced is not so drastic as to result in the neutralization of the effect of the combinations. The samples test at various points in the system and are evaluated as in Test Procedure 2 and should establish the properties of the combination at specific treatment levels to inhibit the growth of microorganisms of the sample tested.

EXAMPLE XIV

In order to ascertain whether the inventive combinations are effective in controlling fungi, evaluations are made following the procedure described by Shema et al, "JOURNAL FOR THE TECHNICAL ASSOCIATION OF THE PULP AND PAPER INDUSTRY." 36, 20A-30A, 1953. The procedure described generally entails incorporating the biocide under test in a nutrient substrate such as agar, malt, etc. and pouring the resulting medium in a Petri dish and allowing the medium to solidify. A button of fungus inoculum is placed on the surface of the solidified medium and the medium is incubated for a period of 14 days. After the period, the diameter of the colony is measured and compared with the diameter of the button of inoculum originally placed upon the surface. If there is no increase in the diameter, the growth of the fungus is considered to be completely inhibited and the treatment level which effectuates this is considered the inhibitory concentration.

The fungi species which is utilized as the test microorganism to evaluate the efficacy of the present combinations are *Penicillium expansum* and *Aspergillus niger.* The study should reveal that the combination of this invention inhibits the growth of *Penicillium expansum* and of *Aspergillus niger.*

EXAMPLE XV

A field test is conducted as in Example XII except a cooling tower is sampled.

EXAMPLE XVI

The effectiveness of the oxidizing agent potassium monopersulfate (OXONE) in combination with several microbiocides is actually unexpected in nature, as shown by the following tests with a sample of microorganisms taken from an air washer in a textile mill located in Talladega, Alabama.

TABLE XVI

Study for combinations of microbiocides and OXIDANTS AGAINST BACTERIA.

TREATMENT	RATIO	TREATMENT LEVEL	EASICULT TEST RESULTS (bacteria/ml)
Control	0/0	0	10^6
a: BAC-20 : OXONE	100/0	10/0	10^2
b: BAC-20 : OXONE	0/100	0/10	10^3
c: BAC-20 : OXONE	50/50	5/5	10^1

The above results show unexpected behavior in that the 50/50 weight mixture of BAC-20 and OXONE showed a greater kill than an equal weight of either the Microbiocide (BAC-20) or the oxidant OXONE by itself.

Similarly, in the same test designed to establish a range of effective combinations of MICROBIOCIDE 76 and OXONE we show the following results:

d: MICROBIOCIDE 76 : OXONE	100/0	40/0	5×10^5
e: MICROBIOCIDE 76 : OXONE	90/10	36/2	5×10^5
f: MICROBIOCIDE 76 : OXONE	75/25	30/5	10^3
g: MICROBIOCIDE 76 : OXONE	50/50	20/10	10^3
h: MICROBIOCIDE 76	25/75	10/15	10^4
i: MICROBIOCIDE 76 : OXONE	10/90	4/18	5×10^5
j: MICROBIOCIDE 76 : OXONE	0/100	0/20	5×10^5

In the above series, "d" through "j", the ratio is the percent of effective concentration of the MICROBIOCIDE 76 and OXONE respectively. Tests "f" and "g" in which the Microbiocide and oxidant were combined in less than the effective concentration of either shows the combination as being more effective than either component alone (see "d" and "j").

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as in the art are intended to be included within the scope of the following claims.

What is claimed is:

1. A microbiocidal combination of materials to be added to a system at the time of use comprising:
   (i) a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and
   (ii) a microbiocidally effective amount of microbicide for inhibiting the growth of microorganisms selected from the group consisting of 2,2-dibromo-3-nitrilopropion- amide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-iosthiazolin-3-one, tetrahydro-3, 5,-dimethyl-2H,1,3,5-thiadiazine-2-thione, and sodium dimethyldithiocarbamate/disodium ethylene bis dithiocarbmate.

2. A method of controlling the growth and deposition of slime-forming organisms in flowing water systems which comprises adding to the flowing water in said flowing water system the combination as defined in claim 1.

3. A method of controlling the growth and deposition of algae in flowing water systems which comprises adding to the flowing water in said flowing water system the combination as defined in claim 1.

4. A method of controlling the growth and deposition of slime-forming organisms in aqueous systems which comprises adding to said aqueous systems the combination as defined in claim 1.

5. A method of controlling slime in pulp and paper mill water systems which comprises adding to said water systems the combination as defined in claim 1.

6. A method of controlling the growth and proliferation of sulfate reducing bacteria as well as species of slime forming microorganisms in petroleum operation systems, which comprises adding to said petroleum operation systems the combination as defined in claim 1.

7. A method of controlling the growth and proliferation of algae, bacteria, and fungi in fresh water which comprises adding to said fresh water the combination as defined in claim 1.

8. A method of controlling the growth and profileration of algae, bacteria and fungi in cooling water which comprises adding to said cooling water the combination as defined in claim 1.

9. A method of controlling the growth and deposition of slime forming microorganisms in water which comprises adding to the water:
   (i) a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and
   (ii) a microbiocidally effective amount of a microbicide for inhibiting the growth of microorganisms selected from the group consisting of 2,2-dibromo-3-nitrilopropion- amide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one, tetrahydro-3, 5,dimethyl-2H,1,3,5-thiadiazine-2-thione and sodium dimethyldithiocarbamate/disodium ethylene bis dithiocarbamate.

10. The method of claim 9, comprising a ratio of at least 10 parts of the microbiocide and 90 parts by weight of the oxidant to 90 parts by weight of the microbiocide/10 parts by weight of the oxidant.

11. A microbiocidal combination of materials to be added to a system at the time of use comprising:
   (i) a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate,
   (ii) a microbiocidally effective amount of a microbicide for inhibiting the growth of microorganisms selected from the group consisting of 2,2-dibromo-3-nitrilopropion- amide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazoline-3-one, tetrahydro-3, 5,dimethyl-2H,1,3,5-thiadiazine-2-thione, and sodium dimethyldithiocarbamate/disodium ethylene bis dithiocarbamate, and
   (ii) a surfactant.

12. The combination of claim 11, comprising:
   (i) at least 5 ppm of said oxidant
   (ii) at least 2.5 ppm of said microbiocide, and
   (iii) about 400 ppm of said surfactant.

13. The combination of claim 11, comprising 2.5 ppm to 45 ppm of said oxidant and 2.5 ppm to 45 ppm of said microbiocide.

14. The combination of claim 11, wherein the surfactant is a fluorinated surfactant.

15. The combination of claim 11, further comprising an anti-corrosive material.

16. The combination of claim 15, wherein the anti-corrosive material is tetrapotassium pyrophosphate or sodium tripolyphosphate.

17. The combination of claim 1, wherein said oxidant of group (i) is selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and said microbiocide of group (ii) is 2,2-dibromo-3-nitrilopropionamide.

18. The combination of claim 1, wherein said oxidant of group (i) is potassium monopersulfate and said mi-

crobiocide of group (ii) is 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one.

19. The combination of claim 1, wherein said oxidant of group (i) is hydrogen peroxide and said microbiocide of group (ii) is methylene bis thiocyanate.

20. The combination of claim 1, wherein said oxidant of group (i) is hydrogen peroxide and said microbiocide of group (ii) is tetrahydro-3-,5-dimethyl-2 H, 1, 3, 5-thiadiazine-2-thione.

21. The combination of claim 1, wherein said oxidant of group (i) is sodium percarbonate and said microbiocide of group (ii) is 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one.

22. A microbiocidal combination of materials to be added to a system at the time of use comprising a ratio of at least 10 parts of microbiocide and 90 parts by weight of oxidant to 90 parts by weight of microbiocide to 10 parts by weight of oxidant, wherein:

(i) said oxidant is selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and

(ii) said microbiocide is selected from the group consisting of 2,2-dibromo-3-nitrilopropionamide, methylene bis thiocyanate, 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one, tetrahydro-3,5,dimethyl-2H,1,3,5-thiadiazine-2-thione, and sodium dimethyldithiocarbamate/-disodium ethylene bis dithiocarbamate.

23. A method of controlling the growth and deposition of algae, bacteria and fungi in a swimming pool which comprises adding to said pool the combination as defined in claim 1.

24. The combination of claim 1, wherein said oxidant of group (i) is OXONE.

25. The combination of claim 1, wherein said oxidant of group (i) is hydrogen peroxide and said microbiocide of group (ii) is sodium dimethyldithiocarbamate/-disodium ethylene bis dithiocarbamate.

26. A method of controlling the growth and deposition of slime forming microorganisms in water which comprises adding to the water:

a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and

a microbiocidally effective amount of a microbicide for inhibiting the growth of microorganisms.

27. A microbiocidal combination of materials to be added to a system at the time of use comprising:

a microbiocidally effective amount of an oxidant for inhibiting the growth of microorganisms selected from the group consisting of potassium monopersulfate, sodium perborate, hydrogen peroxide and sodium percarbonate, and

a microbiocidally effective amount of a microbicide for inhibiting the growth of microorganisms.

28. A method of controlling the growth and deposition of slime forming microorganisms in water which comprises adding to the water:

a microbiocidally effective amount of an oxidant capable of releasing nascent oxygen for inhibiting the growth of microorganisms, and

a microbiocidally effective amount of a microbicide for inhibiting the growth of microorganisms.

29. A microbiocidal combination of materials to be added to a system at the time of use comprising:

a microbiocidally effective amount of an oxidant capable of releasing nascent oxygen for inhibiting the growth of microorganisms, and

a microbiocidally effective amount of a microbicide for inhibiting the growth of microorganisms.

* * * * *

US006143909A

United States Patent [19]

Hoard et al.

[11] Patent Number: 6,143,909

[45] Date of Patent: Nov. 7, 2000

[54] **SELECTIVE EPOXIDATION PROCESS FOR PREPARING PHARMACEUTICAL COMPOUNDS**

[75] Inventors: **David Warren Hoard**, Greenwood; **Eric David Moher**, Indianapolis, both of Ind.; **Vinod Francis Patel**, Waltham, Mass.; **Bryan Hurst Norman**, Indianapolis, Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **09/380,005**

[22] PCT Filed: **Feb. 25, 1998**

[86] PCT No.: **PCT/US98/03667**

§ 371 Date: **Aug. 24, 1999**

§ 102(e) Date: **Aug. 24, 1999**

[87] PCT Pub. No.: **WO98/38164**

PCT Pub. Date: **Sep. 3, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/039,114, Feb. 26, 1997, and provisional application No. 60/039,116, Feb. 26, 1997.

[51] **Int. Cl.**[7] **C07D 303/38**; C07D 301/02

[52] **U.S. Cl.** **549/549**; 549/519

[58] **Field of Search** 549/519, 549

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,845,085	7/1989	Sesin	31/395
4,845,086	7/1989	Sesin	31/395
4,868,208	9/1989	Sesin et al.	514/475
4,946,835	8/1990	Hirsch et al.	514/183
5,256,802	10/1993	Macke et al.	549/519
5,843,992	12/1998	Nomura et al.	514/475
5,998,637	12/1999	Furui et al.	549/518
6,025,506	2/2000	Kaptein et al.	549/519
6,103,666	1/2000	Jew et al.	514/475

FOREIGN PATENT DOCUMENTS

WO 95/17093	12/1994	WIPO .
WO 96/40184	3/1996	WIPO .
WO 96/39829	6/1996	WIPO .
WO 97/07798	8/1996	WIPO .
WO 97/08334	8/1996	WIPO .
WO 97/23211	12/1996	WIPO .
WO 98/08505	8/1997	WIPO .
WO 98/08506	8/1997	WIPO .
WO 98/08829	8/1997	WIPO .

OTHER PUBLICATIONS

Russell A. Barrow, et al., Total Synthesis of Cryptophycins. Revision of The Structures of Cryptophycins A and C. *J. Am. Chem. Soc.* (1995), 117, 2479–2490.

Robert E. Schwartz, et al. Pharmaceuticals From Cultured Algae. *Journal of Industrial Microbiology,* 5 (1990) 113–123.

Motomasa Kobayashi, et al. A total synthesis of Arenastatio A, an extremely potent cyctotoxic eepsipeptide, from the Okinawan marine sponge *Dysidea Arenaria, Chem. Pharm. Bull.* (1994) 42 (11); 2394–2396.

Charles D.Smith, et al., Cryptophycin: A new Antimicrotubule Agent Active Against Drug–Resistant Cells. *Cancer Research* 54, 3779–3784, Jul. 15, 1994.

Golakoti Trimurtulu, et al., Total Structures of Cryptophycins, Potent Antitumor Depsipeptides from the Blue–Green Alga Nostoc SP. Strain GSV 224. *J. Am. Chem. Soc.* 1994, 116, 4729–4737.

Kristen Kerksiek, et al., Interaction of Cryptophycin 1 With Tubulin and Microtubules. *FEBS Letters* 377 (1995) 59–61.

Motomasa Kobayashi, et al. Improved Total Synthesis and Structure–Activity Relationship of Arenastatin A, A Potent Cytotoxic Spongean Depsipeptide. *Chem. Pharm. Bull* 43 (9) 1598–1600 (1995).

Trimurtulu Golakoti, et al. Structure Determination, Conformational Analysis, Chemical Stability Studies, and Antitumor Evaluation of the Cryptophycins. Isolation of 18 new Analogs From Nostoc SP. Strain GSV 224. *J. Am. Chem. Soc.,* 1995, 117, 12030–12049.

Ruoli Bai, et al. Characterization of The Interaction of Cryptophycin 1 With Tubulin: Binding in The Vinca Domain, Competitive Inhibition of Dolastatin 10 Binding, and an Unusual Aggregation Reaction. *Cancer Research* 56, 4398–4406, Oct. 1, 1996.

Gregorz M. Salamonczyk, et al. Total Synthesis of Cryptophycins via A Chemoenzymatic Approach. *J. Org. Chem.,* 1996, 61, 6893–6900.

Rabindra Rej, et al. total synthesis of cryptophycins and their 16–(3–phenylacryloyl) derivatives. *J. Org. Chem.* 1996, 61, 6289–6295.

Richard E. Moore, et al. The Search for New Antitumor Drugs From Blue–Green Algae. *Current Pharmaceutical Design,* 1996, 2, 317–330.

T.H. Corbett, et al. Preclinical Anticancer Activity of Cryptophycin–8. *Journal of Experimental Therapeutics and Oncology,* vol. 1, No. 2, Mar. 1996, pp. 95–108.

Syed M. Ali, et al. Formal Syntheses of cryptophycin A and Arenastatin A. *Tetrahedron Letters,* vol. 38, No. 10, pp. 1703–1706, 1997.

Gottumukkala V. Subbaraju, et al., Three New Cryptophycins from Nostoc SP. GSV 224, *J. Nat. Prod.,* 1997, 60, 302–305.

Primary Examiner—Deborah C. Lambkin
Attorney, Agent, or Firm—John H. Engelmann

[57] **ABSTRACT**

Cryptophycin compounds possessing a β-epoxy moiety may be made with high stereoselectivity at various steps in the overall synthetic process. This invention also provides novel intermediates useful in preparing Cryptophycin compounds.

40 Claims, No Drawings

SELECTIVE EPOXIDATION PROCESS FOR PREPARING PHARMACEUTICAL COMPOUNDS

Provisional Application No. 60/034,114 Feb. 26, 1997.
Provisional Application No. 60/034,116 Feb. 26, 1997.
This appln is a 371 of PCT/US98/03667 Feb. 25, 1998.

BACKGROUND OF THE INVENTION

Neoplastic diseases, characterized by the proliferation of cells not subject to the normal control of cell growth, are a major cause of death in humans and other mammals. Clinical experience in cancer chemotherapy has demonstrated that new and more effective drugs are desirable to treat these diseases. Such clinical experience has also demonstrated that drugs which disrupt the microtubule system of the cytoskeleton can be effective in inhibiting the proliferation of neoplastic cells.

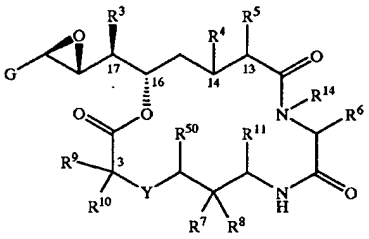
Cryptophycin compounds can now be prepared using a total synthetic process; however, many of the useful cryptophycin compounds contain a labile epoxide group. Barrow, R. A. et al., *J. Am. Chem. Soc.* 117, 2479 (1995). Applicants have discovered that the beta-epoxide can be particularly desired. However, in the Barrow et al. synthesis of some of the cryptophycin compounds of formula (I) below, the epoxidation is performed in the last step which provides only a 2:1 selectivity for the desired epoxide. Furthermore, the diasteromers are difficult to separate at this stage. While it would be desirable to epoxidize an earlier intermediate in the process, epoxides are sensitive to a number of reaction conditions. Moreover, there remains a need for processes with greater stereoselectivity to avoid difficult diastereomeric separations.

The present invention provides a much desired novel and efficient method for preparing cryptophycin compounds having an epoxide functionality. The epoxidation is selective and may be employed at various steps in the overall synthetic process.

BRIEF SUMMARY OF THE INVENTION

The present invention relates to a process for preparing a compound of the formula

(I)



wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^7 and R^8 are each independently hydrogen or C_1–C_6 alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, C_1–C_6 alkyl, C_2–C_6 alkenyl, C_2–C_6 alkynyl, —$(CH_2)_m$—$(C_3$–$C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or C_1–C_6 alkyl;

R^{11} is hydrogen, C_1–C_6 alkyl, phenyl or benzyl;

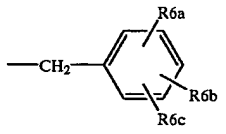
R^{14} is hydrogen or C_1–C_6 alkyl;

R^{50} is hydrogen or (=O);

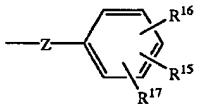
Y' is CH, O, NH, SO, SO_2 or $(C_1$–$C_3)$alkylamino;

R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

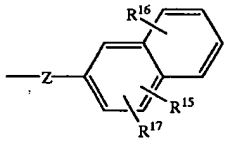
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, halo or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1$–$C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, $(C_1$–C_6 alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or C_1–C_6 alkyl;

R^{23} is hydrogen or $(C_1$–$C_3)$alkyl;

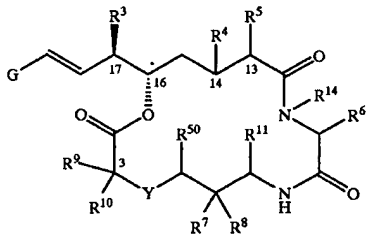
Z is —$(CH_2)_n$— or $(C_3$–$C_5)$cycloalkyl;

n is 0, 1, or 2; and

Z' is an aromatic or substituted aromatic group; or a pharmaceutically acceptable salt thereof;

comprising epoxidizing a compound of the formula

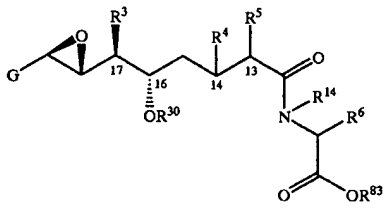
(4)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14} and R^{50} are as defined above and Y is Y' or S; with an oxidant and a chiral ketone to form a compound of formula (I); and optionally forming a pharmaceutically acceptable salt thereof.

This invention further comprises a process for preparing a compound of the formula

(II)



wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

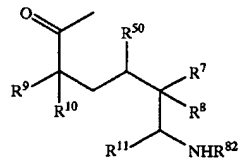
Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^{83} is hydrogen, C_1–C_6 alkyl, trichloroethyl, or $—CH_2SR^{81}$;

R^{30} is hydrogen, an alcohol protecting group, or a group of the formula



R^7 and R^8 are each independently hydrogen or C_1–C_6 alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, C_1–C_6 alkyl, C_2–C_6 alkenyl, C_2–C_6 alkynyl, $—(CH_2)_m—(C_3$–$C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or C_1–C_6 alkyl;

R^{11} is hydrogen, C_1–C_6 alkyl, phenyl or benzyl;

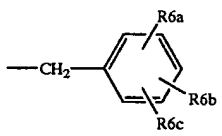
R^{14} is hydrogen or C_1–C_6 alkyl;

R^{50} is hydrogen or (=O);

Y is CH, O, NR^{12}, S, SO, SO_2, wherein R^{12} is H or C_1–C_3 alkyl;

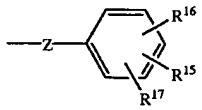
R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

(IA)

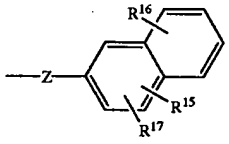


-continued

(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, $(C_1$–$C_6)$alkyl, halo $NR^{18}R^{19}$ or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1$–$C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, $(C_1$–C_6 alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or C_1–C_6 alkyl;

R^{23} is hydrogen or $(C_1$–$C_3)$alkyl;

Z is $—(CH_2)_n—$ or $(C_3$–$C_5)$cycloalkyl;

n is 0, 1, or 2; and

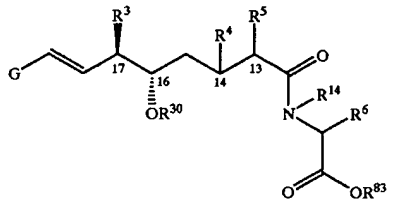
Z' is an aromatic or substituted aromatic group;

R^{81} is C_1–C_6 alkyl, C_3–C_8 cycloalkyl, phenyl or benzyl; and

R^{82} is a base labile protecting group; or a pharmaceutically acceptable salt thereof; with the proviso that when R^{83} is $—CH_2SR^{81}$, R^{30} is not hydrogen or an alcohol protecting group; with the further proviso that when R^{83} is trichloroethyl, R^{30} is not an alcohol protecting group;

comprising epoxidizing a compound of the formula

(5)



wherein G, R^3, R^4, R^5, R^6, R^{14}, R^{30} and R^{83} are as defined above; with an oxidant and a chiral ketone to form a compound of formula (II); and optionally forming a pharmaceutically acceptable salt thereof. The compounds of formula (II) are useful as intermediates in preparing compounds of formula (I).

This invention further comprises the novel compounds of formulae (IId), (18) and (19), disclosed herein, useful in the preparation of compounds of formula (I).

DETAILED DESCRIPTION OF THE INVENTION

As used in the application:

(a) the designation "▬►" refers to a bond that protrudes forward out of the plane of the page;

(b) the designation "||||···" refers to a bond that protrudes backward out of the plane of the page; and

(c) the designation "—" refers to a bond for which the stereochemistry is not designated.

As used herein, the term "pharmaceutically acceptable salt" refers to either acid addition salts or base addition salts.

The expression "pharmaceutically acceptable acid addition salt" is intended to apply to any non-toxic organic or inorganic acid addition salt of the compounds of formula I or any of its intermediates. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulphuric, and phosphoric acid and acid metal salts such as sodium monohydrogen orthophophate, and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include the mono-, di- and tricaboxylic acids. Illustrative of such acids are for example, acetic, glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic, 2-phenoxy-benzoic, and sulfonic acids such as p-toluenesulfonic acid, methane sulfonic acid and 2-hydroxyethane sulfonic acid. Such salts can exist in either hydrated or substantially anhydrous form.

The expression "pharmaceutically acceptable basic addition salts" is intended to apply to any non-toxic organic or inorganic basic addition salts of the compounds of formula I or any of its intermediates. Illustrative bases which form suitable salts include alkali metal or alkaline-earth metal hydroxides such as sodium, potassium, calcium, magnesium or barium hydroxides; ammonia and aliphatic, cyclic or aromatic organic amines such as methylamine, dimethylamine, trimethylamine, diethylamine, triethylamine, isopropyldiethylamine, pyridine and picoline.

As used herein, the term "C_1–C_{12} alkyl" refers to a saturated straight or branched chain hydrocarbon group of from one to twelve carbon atoms. Included within the scope of this term are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, 2-methylbutyl, 3-methylbutyl, hexyl, heptyl, octyl, nonyl, decyl and the like. Included within the term is the term "C_1–C_6 alkyl" which refers to a saturated, unsaturated, straight or branched chain hydrocarbon radical of from one to six carbon atoms. Included within the scope of this term are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, 2-methylbutyl, 3-methylbutyl, hexyl and the like. Included within the terms "C_1–C_{12} alkyl" and "C_1–C_6 alkyl" is the terms "C_1–C_3 alkyl" which refers to a saturated, unsaturated, straight or branched chain hydrocarbon radical of from one to three carbon atoms. Included within the scope of this term are methyl, ethyl, isopropyl, and the like.

"Substituted (C_1–C_6)alkyl" refers to a C_1–C_6 alkyl group that may include up to three (3) substituents containing one or more heteroatoms. Examples of such substituents are OH, NH_2, $CONH_2$, CO_2H, PO_3H_2 and SO_2R^{21} wherein R^{21} is hydrogen, C_1–C_3 alkyl or aryl.

The term "(C_3–C_8)cycloalkyl" refers to a saturated C_3–C_8 cycloalkyl group. Included within this group are cyclopropyl, cyclobutyl, cyclohexyl, cyclooctyl, and the like. A "substituted (C_3–C_8)cycloalkyl group" refers to a (C_3–C_8)cycloalkyl group having up to three C_1–C_3 alkyl, halo, or OR^{21} substituents. The substituents may be attached at any available carbon atom. Cyclohexyl is an especially preferred cycloalkyl group. The term "—$(CH_2)_m$—(C_3–C_5) cycloalkyl" where m is an integer one, two or three refers to a cyclopropyl, cyclobutyl or cyclopentyl ring attached to a methylidene, ethylidene or propylidene substituent.

The term "C_2–C_{12} alkenyl" refers to an unsaturated straight or branched chain hydrocarbon radical of from two to twelve carbon atoms and having from one to three double bonds. Included within the scope of this term are ethenyl, propenyl, isopropenyl, n-butenyl, isobutenyl, pentenyl, 2-methylbutenyl, 3-methylbutenyl, hexenyl, octenyl, nonenyl, decenyl and the like. It is especially preferred that alkenyl have only one double bond.

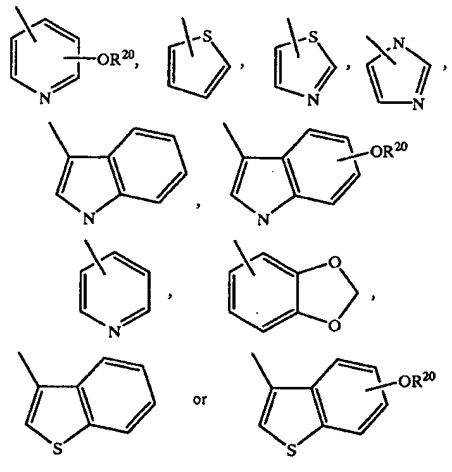
The term "C_2–C_{12} alkynyl" refers to an unsaturated straight or branched chain hydrocarbon radical of from two to twelve carbon atoms and having from one to three triple bonds. Included within the scope of this term are ethynyl, propynyl, isopropynyl, 2-methypropynyl, hexynyl, decynyl, and the like. It is particularly preferred that alkynyl has only one triple bond.

The term "C_1–C_6 alkoxy" refers to a straight or branched alkoxy group containing from one to six carbon atoms, such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, pentoxy, 2-methylpentoxy, and the like. The term "(C_1–C_6 alkoxy)phenyl" refers to a phenyl group substituted with a C_1–C_6 alkoxy group at any available carbon on the phenyl ring.

The term "halo" refers to chloro, bromo, fluoro, or iodo.

The terms "aromatic group" and "heteroaromatic group" refer to common aromatic rings having 4n+2 pi electrons in a monocyclic or bicyclic conjugated system. The term "aryl" refers to an aromatic group, and the term "aralkyl" refers to an aryl(C_1–C_6-alkyl) group. Examples of aromatic groups are phenyl, benzyl and naphthyl. Heteroaromatic groups will contain one or more oxygen, nitrogen and/or sulfur atoms in the ring. Examples of heteroaromatic groups include furyl, pyrrolyl, thienyl, pyridyl and the like. When the aromatic or heteroaromatic groups are substituted, they may have from one to three independently selected C_1–C_6 alkyl, C_1–C_6-alkoxy or halo, substituents. The aromatic groups may be further substituted with trifluoromethyl, $COOR^{57}$ (wherein R^{57} is hydrogen or C_1–C_6 alkyl), PO_3H, SO_3H, SO_2R^{57}, $N(R^{59})$ (R^{60}) (wherein R^{59} is hydrogen or C_1–C_6 alkyl and R^{60} is hydrogen, C_1–C_6 alkyl, BOC or FMOC), —CN, $—NO_2$, $—OR^{57}$, $—CH_2OC(O)(CH_2)_{m'}NH_2$ (wherein m' is an integer 1 to 6) or $—CH_2—O—Si(R^{57})(R^{58})(R^{59})$ (wherein R^{58} is hydrogen or C_1–C_6 alkyl). Especially preferred substituents for the aromatic groups include methyl, halo, $N(R^{59})$ (R^{60}), and $—OR^{57}$. The substituents may be attached at any available carbon atom.

Especially preferred heterocyclic or substituted heterocyclic groups include



wherein R^{20} is hydrogen or C_1–C_6 alkyl.

The term "aryl" refers to an aromatic group of from 6 to 12 carbon atoms, such as phenyl or naphthyl groups wherein said groups are optionally substituted with one, two or three substituents selected from the group consisting of C_1–C_4 alkyl, halo-substituted C_1–C_4 alkyl, halogen or C_1–C_4 alkoxy. The terms "lower alkoyl group" or "C_1–C_5 alkoxy" refers to an alkyloxy radical made up of an oxygen radical bearing a saturated straight or branched chain hydrocarbyl radical of one to five carbon atoms and specifically includes methoxy, ethoxy, propyloxy, isopropyloxy, n-butyloxy, isobutyloxy, sec-butyloxy, tertiary butyloxy, pentyloxy and the like. Specifically included with the scope of the term "aryl" are phenyl, p-toluoyl, p-methoxyphenyl, p-chlorophenyl, naphthyl and the like.

As used herein, the term "heteroaryl" refers to a substituted or unsubstituted heteroaromatic radical which contains one or more non-carbon substituents within the ring, said substituents selected from oxygen, nitrogen or sulfur. The total number of carbon atoms and non-carbon atoms in the ring range from four to twelve atoms. Specifically included with the scope of the term "heteroaryl" are monocyclic conjugated systems such as furyl, pyrrolyl, thienyl, pyridyl, and the like and bicyclic conjugated systems such as indole.

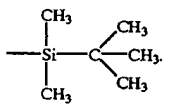
As used herein "epoxide ring" means a three-membered ring whose backbone consists of two carbons and an oxygen atom. As used herein, "aziridine ring" means a three-membered ring whose backbone consists of two carbon atoms and a nitrogen atom. As used herein "sulfide ring" means a three-membered ring whose backbone consists of two carbon atoms and a sulfur atom. As used herein "episulfide ring" means a three-membered ring whose backbone consists of two carbon atoms and a sulfur atom. As used herein "sulfate group" means a five membered ring consisting of a carbon—carbon—oxygen—sulfur—oxygen backbone with two additional oxygen atoms connected to the sulfur atom. As used herein "cyclopropyl ring" means a three member ring whose backbone consists of three carbon atoms. As used herein, "monoalkylphosphate ring" means a five membered ring consisting of a carbon—carbon—oxygen—phosphorous—oxygen backbone with two additional oxygen atoms, one of which bears a lower alkyl group, connected to the phosphorous atom.

As used herein, the term "(=O)" in combination with the carbon on the ring to which it is attached refers to a carbonyl group of the formula

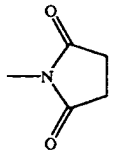
The term "O-aryl" refers to an aryloxy or an aryl group bonded to an oxy moiety.

As used herein, the term "Ph" refers to a phenyl moiety.

As used herein, the term "TBS" refers to tert-butyldimethylsilyl as represented by the formula



As used herein, the term "NHS" refers to a N-hydroxysuccinimide moiety of the formula



As used herein the term "base labile amino protecting group" refers to common amino protecting groups which are known to be base labile. The artisan can consult common works such as Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). See particularly Chapter 7 of Greene. An especially preferred base labile amino protecting group is fluorenylmethoxycarbonyl (Fmoc).

The term "suitable activatable carboxy protecting group" refers to carboxy protecting groups containing activatable ester substituents and are known by one of ordinary skill in the art and disclosed by Greene, T. W., supra. Suitable carboxy protecting groups are those which are activatable ester substituents including N-hydroxy-succinimide, N-hydroxysulfosuccinimide and salts thereof, 2-nitrophenyl, 4-nitrophenyl, 2,4-dichlorophenyl, and the like. An especially preferred activatable carboxy protecting group is N-hydroxy-succinimide (NHS).

As used herein, the term "oxidant" has the meaning associated with the term by the artisan. For example, an oxidant is an agent capable of converting an alkene moiety of a chemical intermediate of this invention to an epoxide moiety. Suitable oxidants include potassium peroxomonosulfate (Oxone), m-CPBA, methyltrioxorhenium (VII), trifluoroper-acetic acid, and magnesium monoperoxyphthalate. A preferred oxidant is potassium peroxomonosulfate (Oxone).

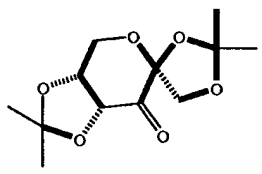
As used herein, the term "chiral ketone" refers to a ketone containing the following general features:

1) the stereogenic centers are close to the reacting center; and

2) the ketone has a fused ring and a a quaternary center α to a carbonyl group; and

3) one face of the ketone is sterically blocked. One especially preferred chiral ketone is of the structure:

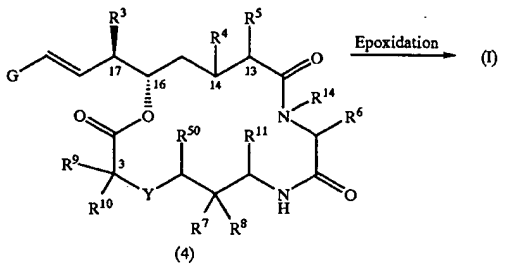
(7)



As used herein the term "alcohol protecting group" can be selected using common works. The term refers to alcohol protecting groups that can be selected from works such as Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). See especially Chapter 2 of Greene. Preferred alcohol protecting groups are selected from silyl and acyl groups. An especially preferred group is tert-butyldimethylsilyl (TBS).

A general synthetic procedure for preparing a compound of formula (I) is set forth in Scheme A. In Scheme A, all substituents unless otherwise indicated, are as previously defined. Reagents, techniques, and procedures used in Scheme A are well known and appreciated by one of ordinary skill in the art.

SCHEME A



In Scheme A, an alkene of formula (4) is epoxidized with a chiral ketone and an oxidant to form a β-epoxide of formula (I).

For example, a compound of formula (4) may be stereoselectively epoxidized to form a β-epoxide of formula (I) using a chiral ketone with an oxidant in the presence of a suitable base such as $NaHCO_3$ using procedures analogous to those disclosed by Tu, Y. et al, *J. Am. Chem. Soc.* 118, 9806 (1996); Wang, Z-X et al. *J. Org. Chem.* 62, 2328 (1997); Wang, Z-X et al., *J. Am. Chem. Soc.* 119, 11224 (1997). Preferred compounds of formula (4) for this reaction include those compounds where G is phenyl, R^3 is methyl, R^4 and R^5 form a second bond, R^{14} is hydrogen, R^{11} is hydrogen, R^{50} is (=O), and Y is O. The preferred oxidant is Oxone and the preferred chiral ketone is the compound of formula (7).

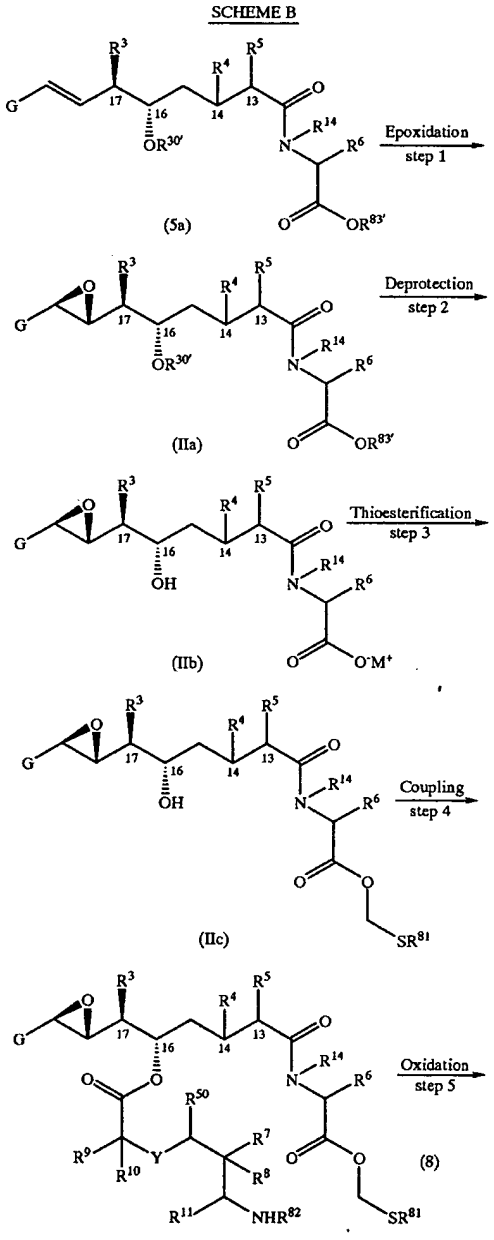
This preferred chiral ketone can be prepared from D-fructose by ketalization and oxidation under routine conditions. For example, the ketalization can be completed using acetone, $HClO_4$, and the process is conducted at about 0° C. For example, the oxidation can be completed using pyridinium chlorochromate at room temperature. These reactions are known in the art; see, for example: Tu, Y. et al, supra. and Wang, Z-X et al. supra. The asymmetric epoxidation can be carried out at a pH within the range of from about 7.0 to about 11.5.

Although it requires about 3–4 equivalents of chiral ketone to obtain conversions of greater than 95% with many cryptophycin intermediates at a pH of about 8.0, it is possible to use less chiral ketone (about 1–2 equivalents) at a pH of about 9.0 or above. Suitable solvents useful for the epoxidation step include H_2O, DMF, glyme, dioxane, CH_3CN, alcohols, THF, EtOAc, halohydrocarbons, chlorobenzene, and toluene, with a CH_3CN/H_2O solvent combination being preferred. Reaction temperatures may range from about −20° C. to about 25° C. with about −10° C. to about 10° C. being preferred. The β-epoxide of formula (I), can be isolated and purified by techniques well known in the art such as extraction, evaporation, chromatography and recrystallization. A preferred stereoselective epoxidation utilizes the chiral ketone of structure (7) to provide a mixture of epoxides (α and β) in the crude product (I) in the ratio of about α:β 1:5. This procedure can also be utilized analogously to obtain the α-epoxide derivative of formula (I).

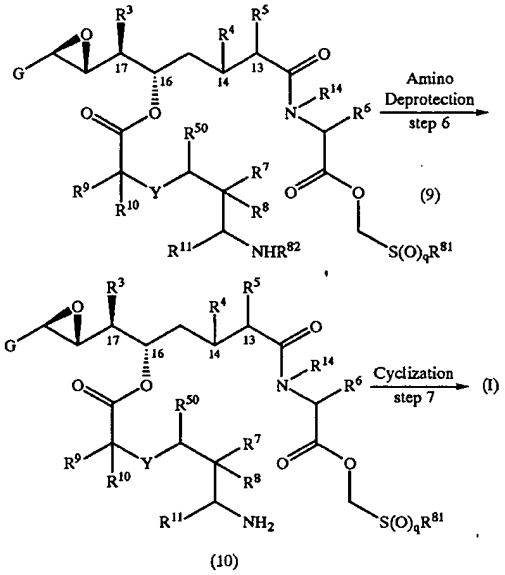
The alkenes of formula (4) are known and may be prepared according to techniques and procedures known in the art. Barrow, R. A. et al. *J. Am. Chem. Soc.* 117, 2479 (1995); PCT Intnl. Publ. No. WO 97/07798, published Mar. 6, 1997, PCT Intnl. Publ. No. WO 96/40184, published Dec. 19, 1996.

A general synthetic procedure for preparing a compound of formula (II), useful as an intermediate for making the β-epoxides of formula (I), is set forth in Scheme B. In Scheme B, $R^{83'}$ is hydrogen or C_1–C_6 alkyl; R^{83} is a base labile protecting group; $R^{30'}$ is an alcohol protecting group; and q is an integer 1 or 2. All other substituents unless otherwise indicated, are as previously defined. Reagents, techniques, and procedures used in Scheme B are well known and appreciated by one of ordinary skill in the art.

SCHEME B



-continued



In Scheme B, step 1, an alkene of formula (5a) is epoxidized with a chiral ketone and an oxidant according to the procedure set forth in Scheme A to form a fragment A-B β-epoxide of formula (IIa).

In Scheme B, step 2, a fragment A-B β-epoxide of formula (IIa) is deprotected with a suitable alkoxy deprotecting agent to form a compound of formula (IIb).

A suitable alkoxy deprotecting agent is one that removes the hydroxy protecting group signified by the $R^{30'}$ substituent while inert to the epoxide moiety of the fragment A-B compound of formula (IIa). Preferred deprotecting agents include basic fluoride sources such as tetrabutylammonium fluoride, pyridinium fluoride, triethylammonium fluoride, cesium fluoride, and the like, with tetrabutylammonium fluoride being preferred. The deprotection reaction takes place in the presence of a suitable organic solvent such as tetrahydrofuran, optionally in the presence of a suitable base, such as sodium bicarbonate ($NaHCO_3$). The reaction takes place in the range of from about 0° C. to about 80° C. with from about 20° C. to about 70° C. being preferred. The reaction is run for a period of time ranging from about 3 to 24 hours. Crude product (IIb) may be used without further purification. Alternatively, the compound of formula (IIb) may be isolated and purified according to procedures well known well known in the art such as extraction, evaporation, chromatography and recrystallization.

In Scheme B, step 3, the compound of formula (IIb) is contacted with a thioester forming agent to provide the thioester of formula (IIc).

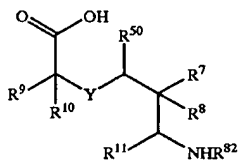
The term "thioester forming agent" encompasses any suitable means or conditions for forming the thioester moiety of formula (IIc). Included within this definition are the conditions set forth and/or analogously described in Ono, N. et al., *Bull. Chem. Soc. Jpn.* 51 (8), 2401 (1978); Ho, Tse-Lok, *Synth. Comm.* 9(4), 267–270 (1979); Narasaka, K. et al., *J. Am. Chem. Soc.* 106 (10), 2954–2960 (1984); L. G. Wade, Jr. et al., *Tetrahedron Lett.* 731–732 (1978); Mora, N. et al., *Tetrahedron Lett.* 34 (15), 2461–2464 (1993); and Dossena, A. et al. *J. Chem. Soc. Perkin Trans. I,* 2737 (1981).

For example, the compound of formula (IIb) may be treated with a sterically hindered alkyl halide, such as tert-butylbromide, and a solvent of the formula $(R^{81})(Me)SO$, wherein R^{81} is as defined above, in the presence of a suitable base, such as sodium bicarbonate ($NaHCO_3$). A preferred solvent for reaction is dimethylsulfoxide (DMSO). Both the sterically hindered alkyl halide and the suitable base are added in a molar excess of about 7.0 to 12.0 in comparison to the compound of formula (IIb). The reaction takes place in the range of from about 0° C. to about 60° C. with from about 10° C. to about 30° C. being preferred. The reaction is run for a period of time ranging from about 1 to 24 hours. Crude product (IIc) may be used without further purification. Alternatively, the thioester of formula (IIc) may be isolated and purified according to procedures well known well known in the art such as extraction, evaporation, chromatography and recrystallization.

In those instances when the substituent $R^{83'}$ is a moiety other than hydrogen, the compound of formula (IIb) must first be carboxy-deprotected. Carboxy-deprotections under basic conditions are known by those of ordinary skill in the art. For example, the compound of formula (IIb) may be treated with a suitable base, such as lithium hydroxide (LiOH) for a period of time sufficient to remove the carboxy protecting group, for example from about 1 to 24 hours.

In Scheme B, step 4, a β-epoxy thioester of formula (IIc) is coupled with a carboxylic acid of the formula

(10a)



wherein R^7, R^8, R^9, R^{10}, R^{11}, R^{50} and R^{82} are as defined above to provide the compound of formula (8).

For example, the carboxylic acid of formula (10a) is dissolved in a suitable organic solvent, such as DMF, glyme, dioxane, THF, CH_3CN, EtOAc, and halohydrocarbons, with dichloromethane being preferred. This solution is then treated with a coupling reagent. Possible coupling reagents include DCC, EDCI, and similar reagents, such as DMAP which activate carboxylic acids towards esterification with alcohols. This solution may then be optionally treated with a suitable base such as solid sodium bicarbonate and then contacted with a β-epoxy thioester of formula (IIc). The concentration of (10a) after these additions should range from about 0.1 M to about 2.0 M. The reaction takes place in the range of from about −30° C. to about 60° C. with from about 10° C. to about 30° C. being preferred. The reaction is run for a period of time ranging from about 0.5 to 12 hours. Crude product (8) may be used without further purification. Alternatively, the compound of formula (8) may be isolated and purified according to procedures well known well known in the art such as extraction, evaporation, chromatography and recrystallization.

In Scheme B, step 5, a β-epoxy thioester of formula (8) is oxidized with a suitable oxidizing agent to provide the sulfone or sulfoxide of formula (9).

A suitable oxidizing agent is an agent capable of converting the sulfide of formula (8) into the sulfone or sulfoxide of formula (9), while inert to the epoxide moiety of the molecule. Suitable oxidizing agents include potassium peroxomonosulfate (Oxone), m-CPBA, methyltrioxorhenium (VII), and magnesium monoperoxyphthalate, with Oxone being preferred.

For example, the sulfide of formula (8) is treated with a suitable base, such as sodium bicarbonate followed by a suitable oxidizing agent, such as Oxone. The reaction is carried out in a suitable solvent, such as acetone, DMF, glyme, dioxane, CH_3CN, alcohols, THF, EtOAc, halohydrocarbons, chlorobenzene, and toluene, with acetone being preferred. Generally, the reaction is carried out at temperatures of from about −30° C. to about 50° C. with from about −10° C. to about 10° C. being preferred. Generally, the reaction requires from about 15 minutes to about 5 hours. Crude sulfone or sulfoxide (9) may be used without further purification. Alternatively, the sulfone or sulfoxide of formula (9) may be isolated and purified according to procedures well known well known in the art such as extraction, evaporation, chromatography and recrystallization.

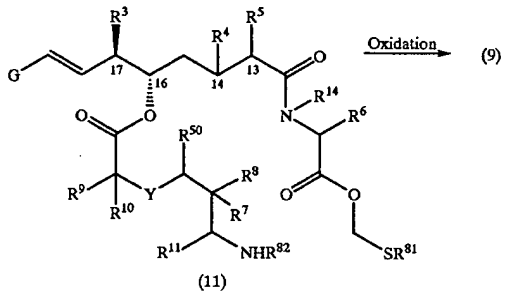
In Scheme B, step 6, the sulfone or sulfoxide of formula (9) is deprotected with a suitable deprotecting agent to provide the amine of formula (10).

A suitable deprotecting agent is an agent capable of removing the base labile substituent R^{82} on the compound of formula (9) while inert to the epoxide moiety of the molecule. Suitable deprotecting agents include bases such as secondary and tertiary amines and inorganic bases, for example, piperidine; morpholine, dicyclohexylamine, p-dimethylaminopyridine, diisopropylethylamine, and the like, with piperidine being preferred. The reaction is carried out in a suitable solvent such as DMF, glyme, dioxane, CH_3CN, alcohols, THF, EtOAc, halohydrocarbons, chlorobenzene, or toluene. Generally, the reaction is carried out at a temperature ranging from about 0° C. to about 120° C. Generally, the reaction requires from about 1 to 72 hours. The compound of formula (I) may be isolated and purified by techniques well known in the art, such as extraction, evaporation, chromatography and recrystallization. Alternatively, the compound of formula (10) is isolated and may be further cyclized with a cyclizing agent to provide a compound of formula (I).

Typically, once the compound of formula (9) is deprotected, it undergoes spontaneous cyclization. However, some particular compounds of formula (9) may require an additional cyclization step. For example, the sulfide of formula (8), although much less active than its oxidized counterpart, upon removal of the base-labile protecting group may be cyclized with a suitable cyclizing agent, such as 2-hydroxypyridine to form a compound of formula (I). For example, the sulfide of formula (8), or alternatively a selected compound of formula (10), is heated in a suitable solvent, such as DMF at about 60° C. for several days in the presence of piperidine and 2-hydroxypyridine. The compound of formula (I) is isolated and purified by techniques well known in the art, such as extraction, evaporation, chromatography and recrystallization.

Alternatively, a compound of formula (9) may be formed according to SCHEME B1. In SCHEME B1, all substituents are as previously defined except where otherwise indicated.

SCHEME B1

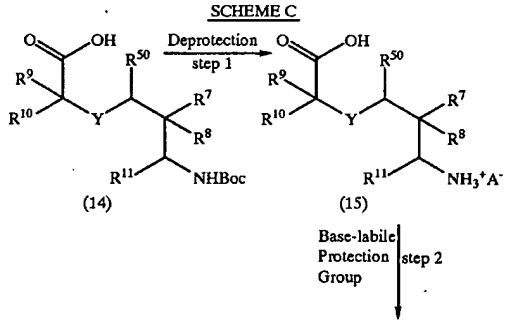


In SCHEME B1, alkene of formula (11) is epoxidized with a chiral ketone and an oxidant according to the procedure set forth in Scheme A to form a compound of formula (9). The alkene of formula (11) may be prepared by one of ordinary skill in the art according to analogously known techninques and procedures. During the epoxidation reaction, the sulfide moiety of compound (11) is oxidized to form the sulfoxide or sulfone moiety of compound (9).

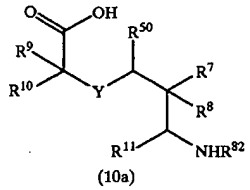
Optionally, on those compounds of formula (I) containing basic or acidic functional groups, pharmaceutically acceptable salts of the compounds of formula (I) may be formed using standard techniques. For example, the free base may be dissolved in aqueous or aqueous-alcohol solution or other suitable solvent containing the appropriate acid and the salt isolated by evaporating the solution. Alternatively, the free base may be reacted in an organic solvent containing the appropriate acid and the salt isolated by evaporating the solution. Further, the free base may be reacted in an organic solvent in which case the salt separates directly or can be obtained by concentration of the solution or in a solvent such as water which is then removed in vacuo or by freeze-drying, or by exchanging the cations of an existing salt for another cation on a suitable ion exchange resin.

A synthetic scheme for making the carboxylic acids of formula (10a) is set forth in Scheme C. The reagents and starting material are readily available to one of ordinary skill in the art. In Scheme C, all substituents, unless otherwise indicated, are as previously defined.

SCHEME C



-continued



(10a)

In Scheme C, step 1, the Boc-protected amine of formula (14) is deprotected to provide the deprotected amine of formula (15).

For example, the deprotection reaction involves the removal of an amino protecting group by techniques and procedures well known and appreciated by one of ordinary skill in the art. The selection, use, and removal of protecting groups are set forth by Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). For example, the Boc-protected amine of formula (14) is dissolved in a suitable acid, such as trifluoroacetic acid or hyrdrochloric acid. Generally, the reaction is carried out at a temperature ranging from about 0° C. to about 60° C. Generally, the reaction requires from about 1 to 24 hours. The deprotected amine of formula (15) may be isolated and purified by techniques well known in the art, such as extraction, evaporation, chromatography and recrystallization.

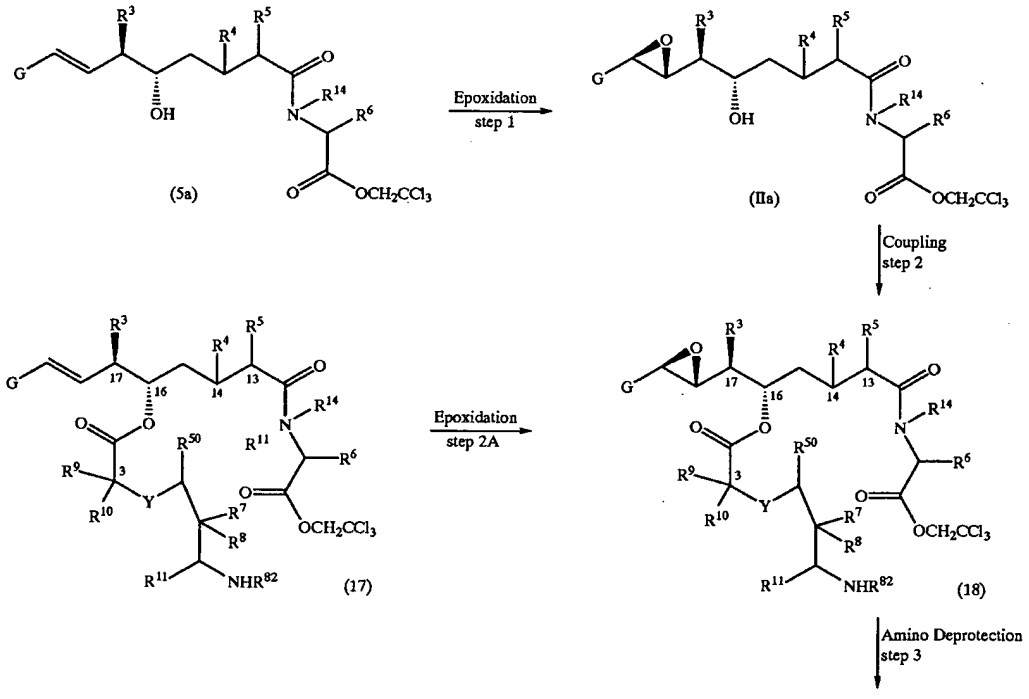
The Boc-protected amine of formula (14) is described in Barrow, R. A. et al. *J. Am. Chem. Soc.* 117, 2479 (1995); PCT Intnl. Publ. No. WO 96/40184, published Dec. 19, 1996; and PCT Intnl. Publ. No. WO 97/07798, published Mar. 6, 1997.

In Scheme C, step 2, the deprotected amine of formula (15) is amino-protected with a base-labile amino protecting group to provide the carboxylic acid of formula (10a).

For example, the protection of an amino group with a base-labile amino protecting group involves the addition of a base-labile amino protecting group by techniques and procedures well known and appreciated by one of ordinary skill in the art. The selection, use, and removal of base-labile amino protecting groups are set forth by Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). A preferred base-labile amino protecting group is Fmoc. For example, to a solution of the deprotected amine of formula (15) in a suitable solvent, such as dioxane, is added a suitable base, such as sodium bicarbonate, followed by a compound of the formula R^{82}-Cl or R^{82}-NHS, such as Fmoc-Cl or Fmoc-NHS succinimide. The mixture may be optionally diluted with a small amount of water and stirred for a period of time ranging from 12 to 48 hours at a temperature ranging from about 0° C. to about 60° C. The mixture may be quenched with a suitable acid, such as hydrochloric acid. The carboxylic acid of formula (10a) may be isolated and purified by techniques well known in the art, such as extraction, evaporation, chromatography and recrystallization.

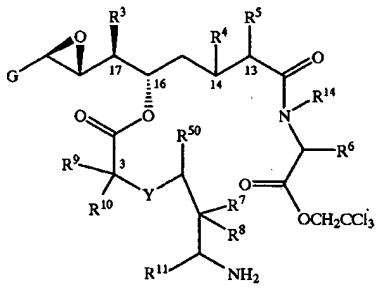
A synthetic scheme for making a compound of formula (II) wherein R^{83} is trichloroethyl is set forth in Scheme D. The reagents and starting material are readily available to one of ordinary skill in the art. In Scheme D, all other substituents, unless otherwise indicated, are as previously defined.

SCHEME D



-continued

(I) Cyclization step 4



In Scheme D, step 1, the alkene of formula (16) is epoxidized with a chiral ketone and an oxidant according to the procedure set forth in Scheme A to form a β-epoxy compound of formula (IId).

In Scheme D, step 2, a β-epoxy compound of formula (IId) is coupled with a carboxylic acid of formula (10a) according to the procedures described in Scheme B, step 4, to form a compound of formula (18).

In Scheme D, step 2A, an alkene of formula (17) is epoxidized with a chiral ketone and an oxidant according to the procedure set forth in Scheme A to form a compound of formula (18).

In Scheme D, step 3, the compound of formula (18) is base-deprotected with a suitable base-deprotecting agent to provide the compound of formula (19).

A suitable base-deprotecting agent is an agent that is capable of removing the base labile substituent R^{82} on the compound of formula (18) while inert to the epoxide moiety of the molecule. Suitable base-deprotecting agents include bases such as secondary and tertiary amines and inorganic bases, for example, piperidine, morpholine, dicyclohexylamine, p-dimethylaminopyridine, diisopropylethylamine, and the like, with piperidine being preferred. These agents are set forth in Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). The reaction is carried out in a suitable solvent such as DMF, glyme, dioxane, CH_3CN, alcohols, THF, EtOAc, halohydrocarbons, chlorobenzene, or toluene. Generally, the reaction is carried out at a temperature ranging from about 0° C. to about 120° C. Generally, the reaction requires from about 1 to 72 hours. The compound of formula (19) may be isolated and purified by techniques well known in the art, such as extraction, evaporation, chromatography and recrystallization.

Typically, once the compound of formula (18) is deprotected, it undergoes spontaneous cyclization to provide a compound of formula (I). However, some particular compounds of formula (18) may yield a compound of formula (19) after deprotection and require an additional ring-closing step as set forth in Scheme D, step 4.

In Scheme D, step 4, the compound of formula (19) is cyclized using a ring-closing agent to provide a compound of formula (I).

The ring-closing reaction may be carried out by intramolecular aminolysis. For example, the compound of formula (19) is treated with a suitable cyclizing agent such as 2-hydroxypyridine analogous to the deprotection and cyclization conditions described in Scheme B, steps 6 and 7 to provide a compound of formula (I).

The pharmaceutically acceptable salts of a compound of formula (I), prepared as described in Scheme D, may optionally be formed according to the procedures described in Scheme B.

Some preferred characteristics of this invention are set forth in the following tabular form wherein the features may be independently selected to provide preferred embodiments of this invention. The invention is in no way limited to the features described below:

A) R^8 is ethyl, propyl, isopropyl, butyl, isobutyl or isopentyl;

B) R^7 is ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, or isopentyl;

C) R^7 is H, R^8 is methyl, R^3 is methyl, and X and Y are not both O;

D) R^3 is ethyl, propyl, isopropyl, butyl, isobutyl, pentyl or isopentyl;

E) R^9 is methyl, ethyl, propyl, butyl, isobutyl, pentyl, or isopentyl;

F) R^{10} is methyl, ethyl, propyl, butyl, isobutyl, pentyl, or isopentyl;

G) Ar is phenyl optionally substituted with a substituent selected from the group consisting of hydrogen, halogen, and simple alkyl;

H) a compound wherein Y is selected from the group consisting of O, NH, S, SO and SO_2;

I) a compound wherein Y is C, R^7, R^8, R^9, and R^{10} are each hydrogen; and R^1 and R^2 form an epoxide;

J) R^7, R^8 are each hydrogen;

K) R^7 and R^8 are each selected from hydrogen or OH;

L) Y is NH;

M) R is selected from the group consisting of methyl, ethyl, n-propyl, and phenyl;

N) X is O and Y is NH;

O) R^4 and R^5 form a double bond;

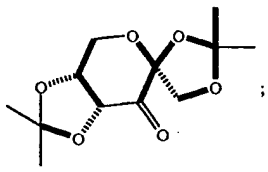
P) R^6 is substituted benzyl wherein one substituent is a halogen and one is an OR^{12} group wherein R^{12} is lower alkyl;

Q) the oxidant is Oxone;

R) the process is utilized to prepare a cryptophycin compound;

S) the epoxidation is selective;

T) the ketone is of the formula:



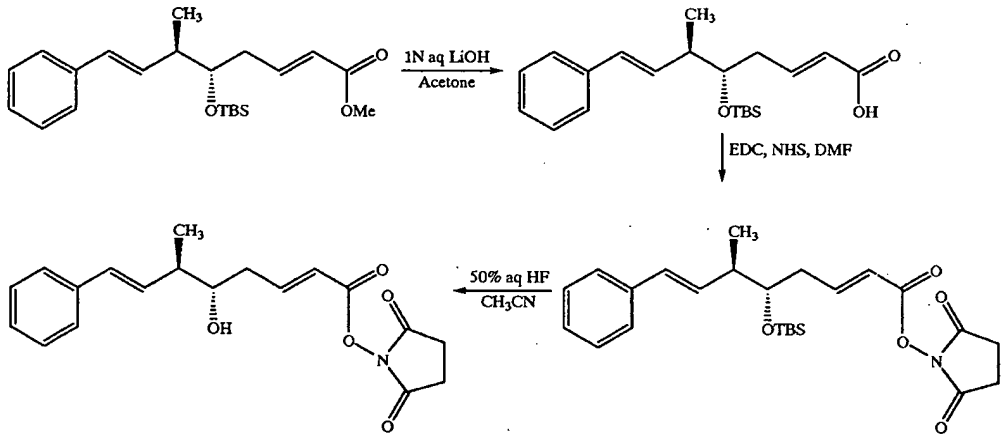
U) R^P is NHS; and

V) R^7 and R^8 are each methyl.

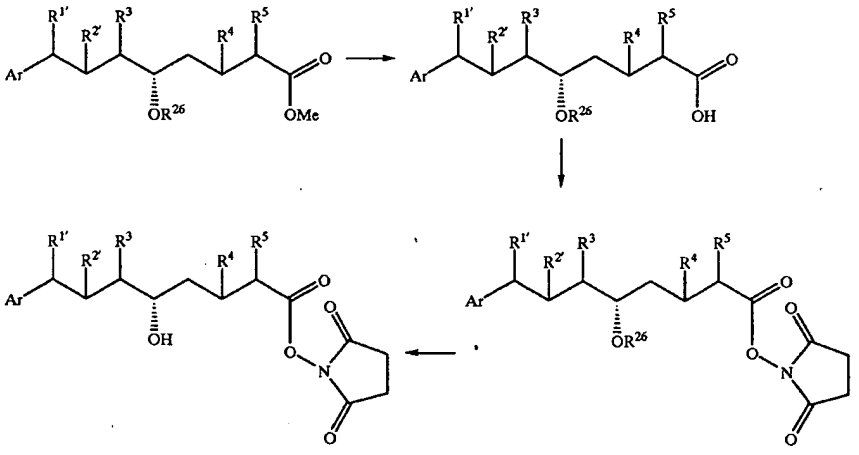
To provide further guidance for the artisan, the following schemes are provided:

As used in Scheme I' and throughout the specification, $R^{1'}$ is halogen, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, phosphate or a protected OH or protected SH group; R^2 is OH or SH; R^{26} is an alcohol protecting group introduced during a portion of the synthetic process to protect an alcohol group which might otherwise react in the course of chemical manipulations, and is then removed at a later stage of the synthesis. Numerous reactions for the formation and removal of such a protecting groups are described in a number of standard works, including, for example, "Protective Groups in Organic Chemistry", Plenum Press, (London and New York, 1973); Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). The skilled artisan can select an appropriate alcohol protecting group particularly with guidance provided from such works. One particularly useful alcohol protecting group is tert-butyldimethylsilyl (TBS).

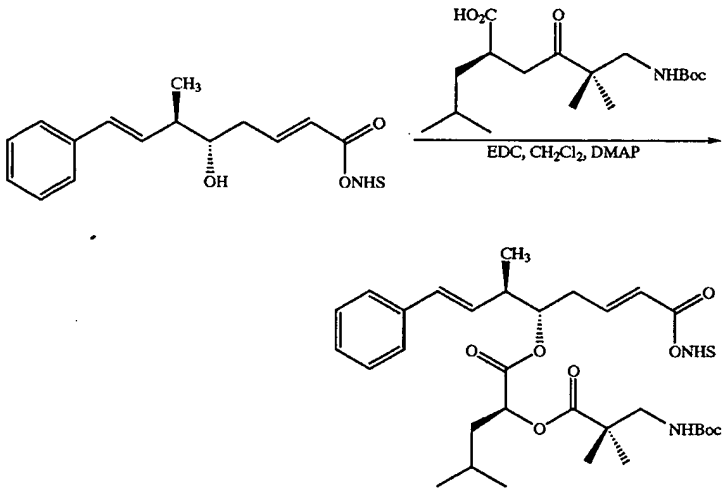
Scheme I



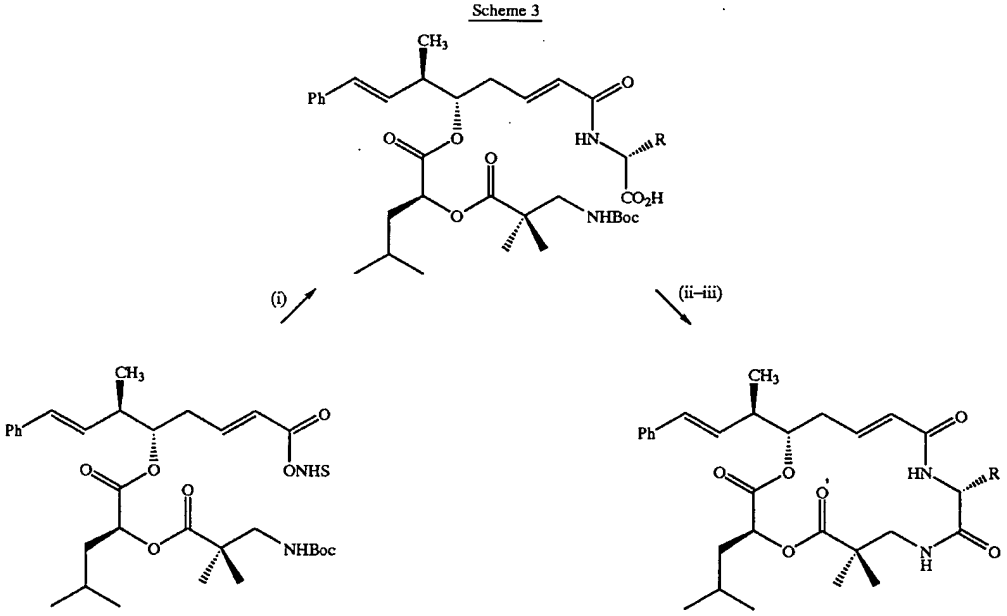
Scheme I'



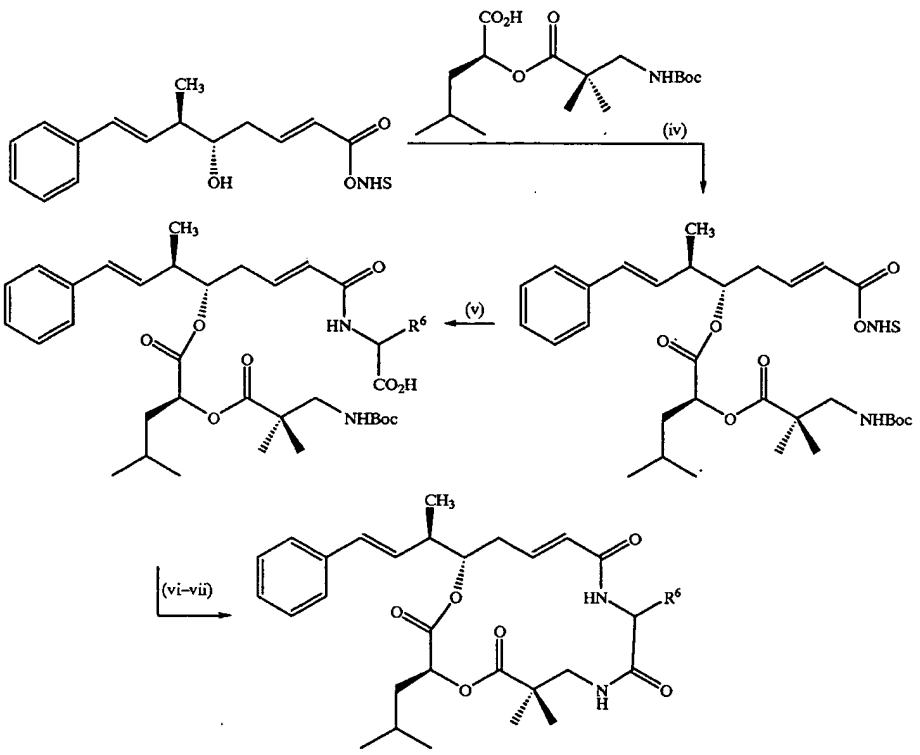
Scheme 2



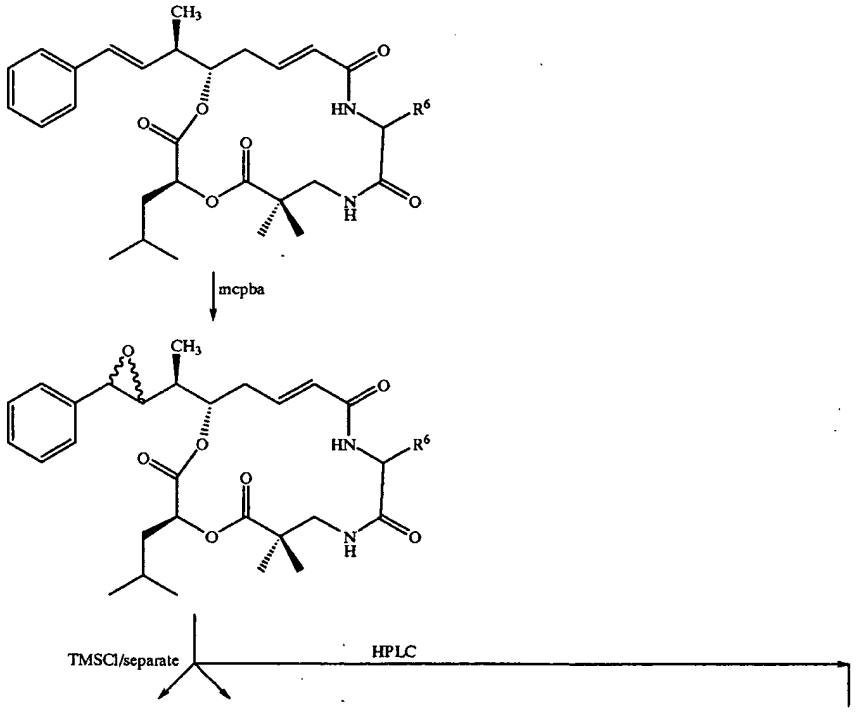
Scheme 3



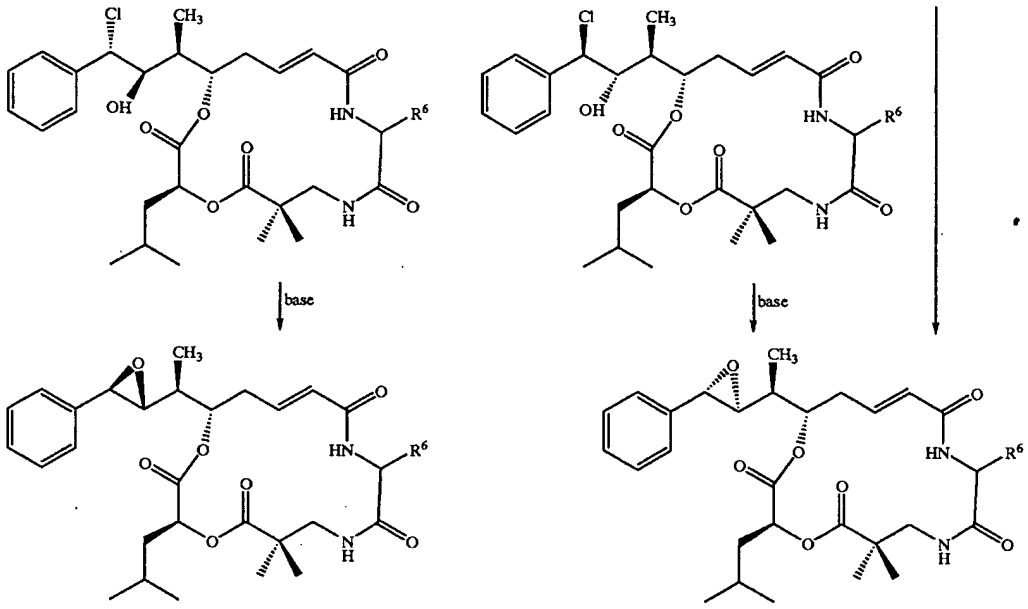
As used in Scheme I' and throughout the specification, $R^{1'}$ is halogen, SH, amino, monoalkylamino, dialkylamino, trialkylammonium, alkylthio, dialkylsulfonium, sulfate, or phosphate; R^2 is OH or SH; R^{26} is an alcohol protecting group introduced during a portion of the synthetic process to protect an alcohol group which might otherwise react in the course of chemical manipulations, and is then removed at a later stage of the synthesis. Numerous reactions for the formation and removal of such a protecting group are described in a number of standard works, including, for example, "Protective Groups in Organic Chemistry", Plenum Press, (London and New York, 1973); Greene, T. W. "Protecting Groups in Organic Synthesis", Wiley (New York, 1981). The skilled artisan can select an appropriate alcohol protecting group particularly with guidance provided from such works. One particularly useful alcohol protecting group is tert-butyldimethylsilyl (TBS). The products of such schemes can be derivatized using standard methods to provide other cryptophycin compounds.



R^6 has the meaning defined supra.



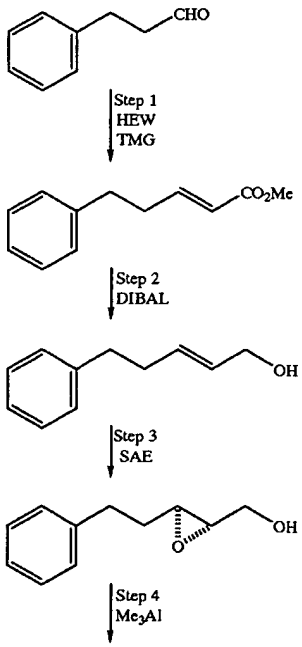
-continued



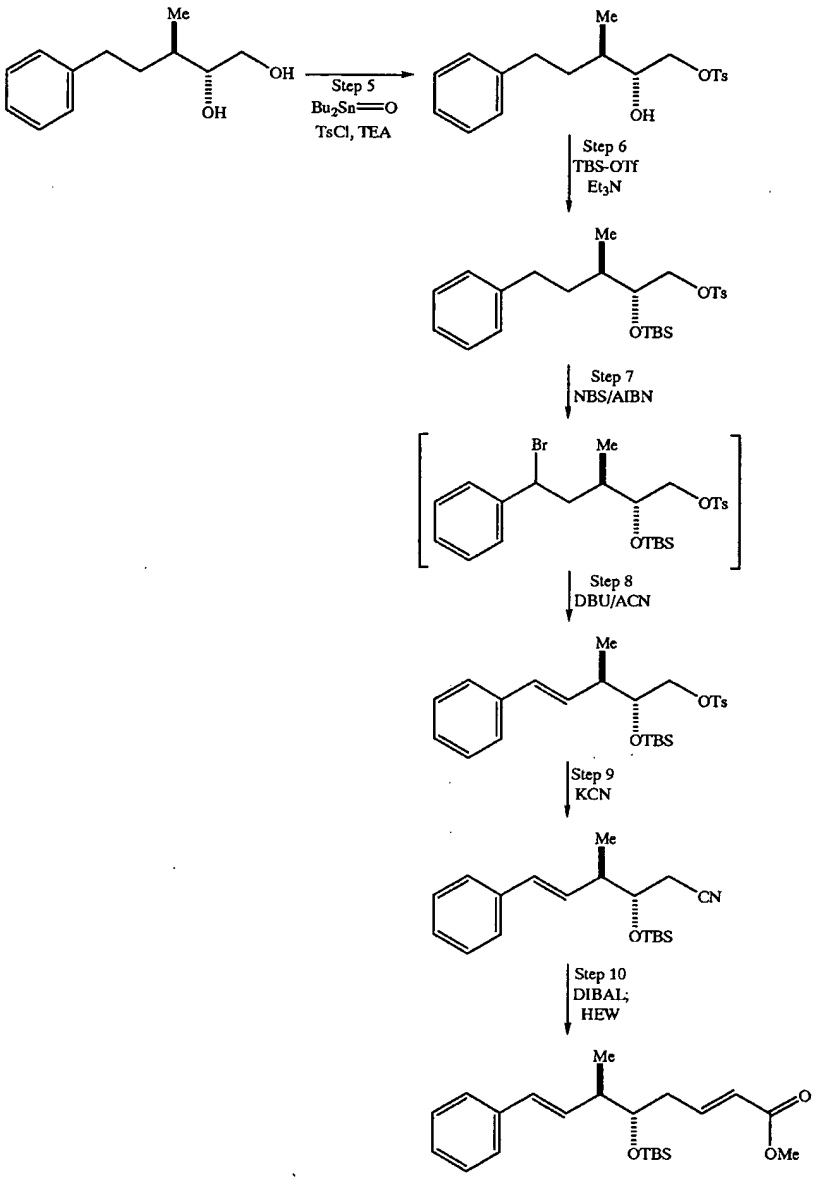
The product of the schemes provided herein can be further derivatized using standard methods to provide further cryptophycin compounds.

The artisan can utilize appropriate starting materials and reagents to prepare desired compounds using the guidance of the previous schemes and following examples.

The ester starting material can be prepared, for example, as follows:



-continued



R^6 has the meaning defined supra.

The scheme for preparing the ester is further explained by the Preparation Section herein which provides one specific application of the scheme for the convenience of the skilled artisan.

The Scheme for preparing the ester is applicable to the Ar substituents claimed herein. The scheme illustration is not intended to limited the synthesis scheme only to the phenyl ring illustrated. Rather, the artisan can broadly apply this process to provide desired starting materials for the compounds claimed herein.

The scheme for preparing the ester is further explained by the Preparation Section herein which provides one specific application of the scheme for the convenience of the skilled artisan.

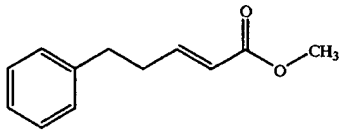
Scheme E for preparing the ester is applicable to the Ar substituents claimed herein. The scheme illustration is not intended to limited the synthesis scheme only to the phenyl ring illustrated. Rather, the artisan can broadly apply this process to provide desired starting materials for use in the processes claimed herein.

The necessary reaction time is related to the starting materials and operating temperature. The optimum reaction time for a given process is, as always, a compromise which is determined by considering the competing goals of throughput, which is favored by short reaction times, and maximum yield, which is favored by long reaction times.

To further illustrate the invention the following examples are provided. The scope of the invention is in no way to be construed as limited to or by the following examples.

Preparation 1

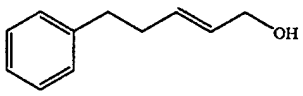
Step 1. Methyl 5-Phenylpent-2(E)-enoate



A solution of trimethyl phosphonoacetate (376 g, 417 mL, 2.07 mol) in THF (750 mL) was stirred at 0° C. in a 3 L 3-neck round bottom flask equipped with a mechanical stirrer and N_2 inlet. To the chilled solution, neat tetramethyl guanidine (239 g, 260 mL, 2.07 mol) was added dropwise via an addition funnel. The chilled clear pale yellow solution was stirred for 25 minutes at 0° C. A solution of hydrocinnamaldehyde (90%, 253 g, 248 mL, 1.9 mol) in THF (125 mL) was added dropwise to the reaction solution slowly. Upon completion of addition, the reaction was stirred for 10 h rising to room temperature. GC indicated a 95:5 ratio of product to starting material. 500 ml of water was added to the reaction vessel and the reaction stirred overnight separating into two layers. The organic layer was isolated and the aqueous layer was extracted with t-BuOMe. The organic layers were combined and dried over $MgSO_4$, then concentrated in vacuo to yield an orange oil. The crude product was distilled at 129° C./0.3 mm Hg yielding 360.5 g, 91.7% yield, of a clear slightly yellow oil.

EIMS m/z 190(13; M+), 159(410, 158(39), 131(90), 130(62), 117(22), 104(12), 95(57), 91(100), 77(21), 65(59); HREIMS m/z 190.0998 ($C_{12}H_{14}O_2$ D –0.4 mnu); UV lmax (e) 210 (8400), 260 (230) nm; IR nmax 3027, 2949, 1723, 1658, 1454, 1319, 1203, 978, 700 cm^{-1}; ^1H NMR d ($CDCl_3$) 7.15–7.3 (Ph-H5;bm), 7.00 (3-H;dt, 15.6/6.6), 5.84 (2-H;dt, 15.6/1.2), 3.70 (OMe;s), 2.76 (5-H2;t, 7.2), 2.51 (4-H2; bdt, 6.6/7.2); ^{13}C NMR d ($CDCl_3$) 166.9 (1), 148.3(3), 140.6 (Ph-1'), 128.4/128.2 (Ph2'/3'/5'6'), 126.1 (Ph 4'), 121.4 (2), 51.3 (OMe), 34.2/33.8 (4/5).

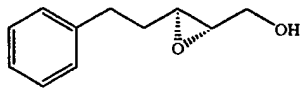
Step 2. 5-phenyl-pent-2-en-1-ol



To a 12 L 4-neck round bottom flask equipped with a thermocouple, mechanical stirrer and N_2 inlet, a solution of enoate ester (310.5 g, 1.5 mol) in THF (1.5 L) was charged and chilled to –71° C. via a i-PrOH/CO_2 bath. To the reaction vessel, was added dropwise DIBAL (2.5 L, 1.5 M in toluene, 3.75 mol) at a rate to maintain the reaction temperature <–50° C. Upon complete addition, the reaction was stirred overnight with the reaction temperature <–50° C. TLC (3:1 Hexanes:EtOAc, SiO_2) indicated absence of starting material after 16 h. The reaction temperature was allowed to raise to –15° C. The reaction was quenched slowly with 1N HCl (150 mL). At this point the reaction setup into a gelatinous solid. A spatula was employed to breakup the the semi-solid and 1N HCl (200 mL) was added making the mixture more fluid. Concentrated HCl (625 mL) was charged to form a two phase system. The layers were separated and the product extracted with t-BuOMe. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield a clear pale yellow oil, 247.8 g. The crude product was distilled at 145° C./0.25 mm Hg yielding 209.7 g, 86.2%.

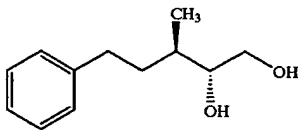
EIMS m/z 162 (1:M+) 144 (16), 129 (7), 117 (9) 108 (6), 92 (17), 91 (100), 75 (5), 65 (12), HREIMS m/z 162, 1049 ($C_{11}H_{14}O$, D –0.4 mmu); UV lmax (e) 206 (9900), 260 (360); IR nmax 3356, 2924, 1603, 1496, 1454, 970, 746, 700 cm^{-1}; ^1H NMR d 7.15–7.3 (Ph-H5;m), 5.70 (3-H;dt, 15.6/6.0), 5.61 (2-H;dt, 15.6/4.8), 4.02 (1-H2;d 4.8), 2.68 (5-H2; t, 7.2), 2.40 (OH;bs), 2.36(4-H2; dt, 6.0/7.2); ^{13}C NMR d141.6 (Ph 1'), 131.8(3), 129.5 (2), 128.3/128.2 (Ph 2'/3'/5'/6'), 125.7 (Ph 4'), 63.3 (1), 35.4/33.8 (4/5).

Step 3. (2S,3S)-2,3-Epoxy-5-phenyl-1-pentanol



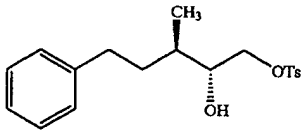
To a 1 L 3 neck round bottom flask equipped with a mechanical stirrer, thermocouple and nitrogen inlet was added CH_2Cl_2 (350 mL), dried 4 Å molecular sieves (30 g) and L-(+)-diethyl tartrate (7.62 g, 0.037 mol). The resulting mixture was cooled to –20° C. and treated with $Ti(O\text{-}i\text{-}Pr)_4$ (9.2 mL, 0.031 mol), followed by the addition of t-butylhydroperoxide (4.0 M in CH_2Cl_2, 182 mL, 0.78 mol) at a rate to maintain the temperature[2] –20° C. Upon complete addition, the reaction mixture was stirred for another 30 min, and then treated with a solution of the allylic alcohol (50 g, 0.31 mol) in CH_2Cl_2 (30 mL) at a rate to maintain the temperature[2] –20° C. The reaction was stirred at the same temperature for 5 h, then filtered into a solution of ferrous sulfate heptahydrate (132 g) and tartaric acid (40 g) in water (400 mL) at 0° C. The mixture was stirred for 20 min, then transferred to a separatory funnel and extracted with t-BuOMe (2x200 mL). The combined organic phase was stirred with 30% NaOH solution containing NaCl, for 1 h at 0° C. The layers were again separated, and the aqueous phase extracted with t-BuOMe. The combined organic phase was washed with brine, dried over $MgSO_4$ and concentrated to yield 52.8 g as an amber oil.

Step 4. (2R, 3R)-2-hydroxy-3-methyl-5-phenylpentan-1-ol



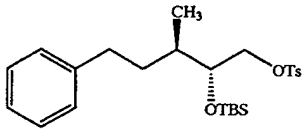
To a 5 L 3 neck round bottom flask equipped with a mechanical stirrer, thermocouple and nitrogen inlet was added hexanes (1 L) and cooled to 0° C. A 2.0M solution of Me_3Al in hexanes (800 mL, 1.6 mol) was added, followed by a solution of the epoxide (120 g, 0.677 mol) in hexanes (250 mL)/CH_2Cl_2 (50 mL) maintaining the temperature below 20° C. Upon complete addition, the cloudy reaction mixture was stirred at 5° C. for 35 min, whereupon a solution of 10% HCl (300 mL) was added dropwise, followed by the addition of concd HCl (350 mL). The layers were separated, and the organic phase was washed with brine and dried over $MgSO_4$. After removal of the volatiles in vacuo, 122.1 gram of an oil was obtained.

Step 5. (2R, 3R)-2-hydroxy-3-methyl-5-phenylpent-1-yl Tosylate



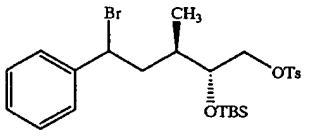
To a 2 L 3 neck round bottom flask equipped with a mechanical stirrer and nitrogen inlet was added the diol (58 g, 0.30 mol), dibutyltin oxide (1.5 g, 0.006 mol, 2 mol %), toluenesulfonyl chloride (57.5 g, 0.30 mol), CH_2Cl_2 (580 mL) and triethylamine (42.0 mL, 0.30 mol). The resulting mixture was stirred at room temperature for 2 h (although the reaction was complete within 1 h), filtered, washed with water and dried over $MgSO_4$. Concentration of the volatiles in vacuo afforded 104.1 gram of a slightly amber oil.

Step 6. (2R, 3R)-2-[(tert-Butyldimethylsilyl)oxy]-3-methyl-5-phenylpent-1-yl Tosylate



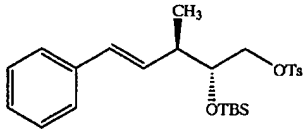
A solution of the tosylate (100 g, 0.29 mol) and triethylamine (81.0 mL, 0.58 mol) in CH_2Cl_2 (1200 mL) was treated with neat TBS-OTf (99 mL, 0.43 mol) dropwise with continued stirring for another 20 min. The reaction was washed twice with brine, dried over $MgSO_4$ and concentrated to dryness. The oil was dissolved in a minimal amount of hexanes and filtered over a silica pad, eluting with hexanes:EtOAc (9:1) to yield a slightly amber oil, 134 g.

Step 7. (2R, 3R,5RS)-2-[(tert-Butyldimethylsilyl)oxy]-3-methyl-5-bromo-5-phenylpent-1-yl Tosylate



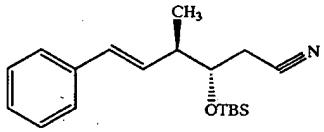
To a 5 L 3 neck round bottom flask equipped with a mechanical stirrer, reflux condenser and nitrogen inlet was added CCl_4 (1680 mL), TBS Ts (140 g, 0.30 mol), NBS (65 g, 0.365 mol) and AIBN (16.5 g, 0.10 mol). The mixture was degassed by evacuation under full vacuum with stirring, and backfilling with nitrogen (3×). The reaction mixture was then heated to reflux, whereupon the color became dark brown. After 15 min at vigorous reflux, the reaction mixture became light yellow, and chromatographic analysis indicated the reaction was complete. After cooling to room temperature, the reaction was filtered and the filtrate concentrated to dryness. The residue was redissolved in hexanes and filtered again, and concentrated to dryness to afford 170.3 gram as an amber oil.

Step 8. (2R, 3R)-2-[(tert-Butyldimethylsilyl)oxy]-3-methyl-5-phenylpent-4(E)-en-1-yl Tosylate



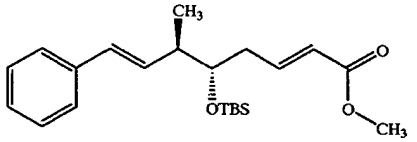
To a 2 L 3 neck round bottom flask equipped with a mechanical stirrer, reflux condenser and nitrogen inlet was added a solution of the bromide (100 g, 0.186 mol) in acetonitrile (700 mL). DBU (83.6 mL, 0.557 mol) was added and the resulting dark brown solution was stirred at reflux for 15 min. After cooling to room temperature, the solvent was removed in vacuo, and the residue digested in CH_2Cl_2 (200 mL) and filtered through a silica pad. The volatiles were again evaporated, and the residue dissolved in EtOAc and washed with water, brine and dried over $MgSO_4$ and concentrated to dryness. Preparative mplc (Prep 500) chromatography afforded the desired unsaturated compound (50.3 g, 60% yield over 4 steps).

Step 9. (3S, 4R)-3-[(tert-Butyldimethylsilyl)oxy]-4-methyl-6-phenylhex-5(E)-en-1-nitrile



The tosylate (50 g, 0.11 mol) was dissolved in DMSO (1 L) and treated with KCN (14.2 g, 0.22 mol) and water (25 mL), and the resulting mixture was stirred at 60° C. under nitrogen for 18 h. After cooling to room temperature, the reaction mixture was partitioned between EtOAc (1 L) and water (1 L). The aqueous phase was extracted with EtOAc (500 mL), and the combined organic phase was washed with brine and dried over Na_2SO_4. Flash chromatography over silica with CH_2Cl_2 afforded the desired nitrile in 92% yield.

Step 10. Methyl (5S, 6R)-5-[(tert-Butyldimethylsilyl)oxy]-6-methyl-8-phenylocta-2(E),7(E)-dienoate



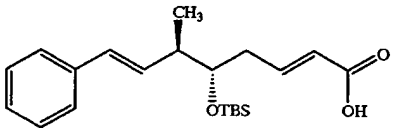
The nitrile (14.67 g, 46.5 mmol) was dissolved in toluene (200 mL) and cooled to −78° C. under nitrogen. A 1.5M solution of DIBAL in toluene (37.2 mL, 55.8 mmol) was added dropwise with vigorous stirring. Upon complete addition, the cooling bath was removed and the reaction was stirred at room temperature for 1 h. The reaction mixture was carefully poured into 1N HCl and the mixture stirred at room temperature for 30 min. The layers were separated, and the organic phase was washed with a saturated aqueous solution of sodium potassium tartrate (2×), brine and dried over Na_2SO_4. The volatiles were removed in vacuo, and the crude pale yellow oil was used directly in the subsequent condensation.

The crude aldehyde from above was dissolved in THF (90 mL) and treated with trimethyl phosphonoacetate (9.03 mL,

55.8 mmol) and tetramethylguanidine (7.0 mL, 55.8 mmol) at room temperature under nitrogen. The reaction mixture was stirred for 16 h, then partitioned between EtOAc (200 mL) and water (100 mL). The aqueous phase was back extracted with EtOAc (100 mL), and the combined organic phase was washed with water, brine and dried over Na_2SO_4. The volatiles were removed in vacuo, and the crude yellow oil (17.0 g) was chromatographed over silica gel with CH_2Cl_2:cyclohexane (1:1 to 2:1) to afford 13.67 grams of the desired ester, 78.5%.

Preparation 2

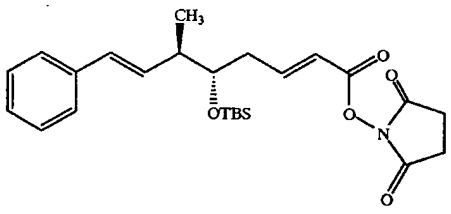
(5S,6R)-5-[(tert-Butyldimethylsilyl)oxy]-6-methyl-8-phenylocta-2(E),7(E)-dienoic acid



Methyl (5S, 6R)-5-[(tert-Butyldimethylsilyl)oxy]-6-methyl-8-phenylocta-2(E),7(E)-dienoate from Preparation 1, step 10 (1.00 g, 2.673 mmol) was dissolved in acetone (44 mL) and then 1N aqueous LiOH (26 mL) added at room temperature. The cloudy mixture was further diluted with acetone (20 mL) and the resulting yellow mixture stirred at room temperature for 23.5 h. The reaction was diluted with diethylether (400 mL) and the organics washed with IN Hcl (120 mL), brine (200 mL) and H_2O (160 mL). The organics were dried ($MgSO_4$) and concentrated in vacuo to leave a yellow oil which was purified by column chromatography (gradient elution: 5% AcOH+20%–40% EtOAc/Hexanes) to give carboxylic acid as a yellow oil (960 mg, 100%).

$[a]_D^{589}$ +87.6° (c 10.5, $CHCl_3$); ^1H NMR ($CDCl_3$) d 7.38–7.19 (m, PhH_5), 7.09 (ddd, J=15.2, 7.6 and 7.9 Hz, 3-H), 6.38 (d, J=16 Hz, 8-H), 6.16 (dd, J=16 and 8 Hz, 7-H), 5.85 (d, J=15.8 Hz, 2-H), 3.81–3.75 (m, 5-H), 2.49–2.37 (m, 6-H, 4-HH'), 1.12 (d, J=6.7 Hz, 6-Me), 0.91 (s, 9H, $SiCMe_3$), 0.065 (s, SiMe), 0.068 (s, SiMe) ppm; IR ($CHCl_3$) l_{max} 2957, 2930, 2858, 1697, 1258, 1098, 838 cm^{-1}; MS (FD) 360.2 (M+,100); Anal. calcd. for $C_{21}H_{32}O_3$ requires: C,69.95; H,8.95%. Found: C,69.19; H,8.39%.

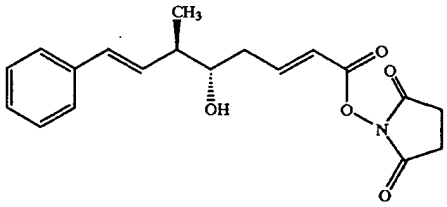
Preparation 3



To a stirred solution of the carboxylic acid of Preparation 2 (720 mg, 2 mmol) in dry dimethylformamide (5.50 mL) was added 1-Ethyl-3-(3-dimethyaminopropyl)carbodiimide (459 mg, 2.4 mmol) and N-hydroxy-succinimide (299 mg, 2.6 mmol) at room temperature. The mixture was stirred for 28 h and then diluted with EtOAc (100 mL) and washed with 1N aqueous HCl (2x50 mL), H_2O (75 mL), dried (MgSO4) and concentrated in vacuo to leave an oil. Crude product was purified by column chromatography (gradient elution: 5–30% EtOAc/Hexanes) to give active ester as a pale yellow oil (724 mg,80%).

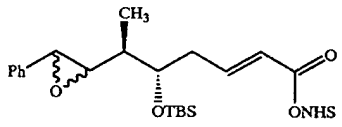
$[a]_D^{589}$ +71.3° (c 10.1, $CHCl_3$); ^1H NMR ($CDCl_3$) d 7.36–7.20 (m, PhH_5, 3-H), 6.38 (d,J=16 Hz, 8-H), 6.14 (dd, J=16.1 and 8.0 Hz, 7-H), 6.03 (d, J=16 Hz, 2-H), 3.79 (q, J=4.3 Hz, 5-H), 2.94 (brs, CH_2CH_2), 2.58–2.42 (m, 6-H, 4-HH'), 1.10 (d,J=6.8 Hz, 6-Me), 0.90 (s, 9H, $SiCMe_3$), 0.05 (s, 6H, $SiMe_2$) ppm; IR ($CHCl_3$) l_{max} 2957, 2931, 2858, 1772, 1741, 1648, 1364, 1254, 1092, 1069, 838 cm^{-1}; MS (FD) 457 (M+,100); Anal. calcd. for $C_{25}H_{35}NO_5$ requires: C,65.61;H,7.71;N,3.06%. Found: C,65.51;H,7.56; N, 3.02%.

Preparation 4



To a stirred solution of active ester of Preparation 3 (2.50 g,5.47 mmol) in CH_3CN (130 mL) was added 48% aqueous HF (15 mL) at 0 C. The solution was stirred at 0 C. for 0.75 h and then at room temperature for 4 h. The reaction was diluted with diethylether (300 mL) and washed with H_2O until the wash was ~pH7. Organics were dried ($MgSO_4$) and concentrated in vacuo to give a yellow residue which was recrystallized from Et2O to give alcohol as white crystals (1.46 g,78%). ^1H NMR ($CDCl_3$) d 7.41–7.20 (m,PhH_5,3-H), 6.48 (d,J=16 Hz,8-H), 6.15–6.07 (m,7-H,2-H), 3.71–3.65 (m,5-H), 2.83 (brs,CH_2CH_2), 2.60–2.33 (m,6-H,4-CH_2), 1.95 (brs, 5-OH), 1.14 (d,J=6.8 Hz,6-Me) ppm; IR u (KBr) 3457,1804,1773,1735,1724,1209,1099,1067,1049,975, 744, 694 cm^{-1}; UV (EtOH) l_{max} 250 (e=20535) nm; MS (FD) 343.2 (M^+,100); $[a]_D$ -57.8° (c 10.56, $CHCl_3$); Anal. calcd. for $C_{19}H_{21}NO_5S$ requires: C,66.46;H,6.16;N,4.08%. Found: C,66.49; H,6.16; N, 4.07%.

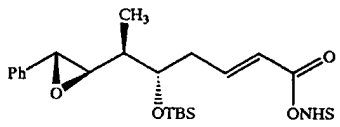
Preparation 5



Acetone (10 mL) was added to a solution of the active ester of Procedure 3 (2.90 g, 6.35 mmol) in dichloromethane (20 mL) and the solution cooled to 0° C. An aqueous solution of oxone (11.7 g, 19 mmol) in H_2O (30 mL) was slowly added to stirred solution of aqueous $NaHCO_3$ (5.3 g, 63.5 mmol) in H_2O (30 mL) (gas evolution observed!). The resulting solution was added to the reaction mixture and stirred at 0° C. for 7 h (tlc- 50% conversion). Further oxone (6 g) and acetone (15 mL) were added and the mixture stirred for 1.5 h (tlc- all SM consumed). The reaction mixture was diluted with H_2O (5 volumes) and product extracted with CH_2Cl_2 (5x100 mL). Combined, dried ($MgSO_4$) organics were concentrated in vacuo to give product as a yellow gummy solid (2.88 g). Tlc and ^1H NMR indicated 90% desired epoxide product (a:b=1:1.62): 10% SM. Crude product was purified by column chromatography (SiO_2:gradient elution:15%–25% EtOAc:Hexanes) to give

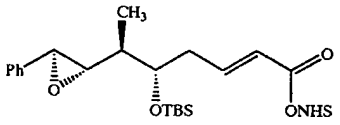
recovered styrene (389 mg, 13%) and epoxide as a yellow oil (2.38 g, 80%). Epoxides (2 g, a:b=1:1.50) were separated by HPLC to give b-epoxide as a white crystalline solid (1.17 g, 59%. 99.8% ee) and a-epoxide as white crystalline solid (0.864 g, 43.2%, 99% ee).

Preparation 6



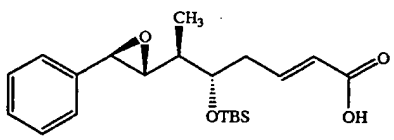
HPLC: C18 reverse phase, flow rate 1 mL/min, 60:40-CH_3CN:H_2O, l=254 nm, b-epoxide Rt=17.2 mins (AUC 1.5); $[a]_D^{589}$ +77.36 (c 1.06, CH_2Cl_2); ^1H NMR ($CDCl_3$) d 7.35–7.24 (m, 6H, ArH_5, 3-H), 6.08 (d, J=15.8 Hz, 2-H), 3.91–3.88 (m, 5-H), 3.70 (s, 8-H), 2.97 (dd, J=6 and 0.9 Hz, 7-H), 2.85 (s, 4H, CH_2CH_2), 2.56–2.51 (m, 4-HH'), 1.78–1.76 (m, 6-H), 1.06 (d, J=6.9 Hz, 6-Me), 0.86 (s, 6H, $SiCMe_3$), 0.05 (s, SiMe), 0.01 (s, SiMe) ppm; IR ($CHCl_3$) u 2957, 2931, 1742, 1773, 1200, 1069, 839 cm^{-1}; UV (EtOH) l_{max} 217 (e=21180) nm; MS (FD) m/z 474 (M^+, 10), 416 ([M-CMe_3]$^+$, 100); Anal. calcd. for $C_{25}H_{35}NO_6$ requires: C, 63.40; H, 7.45; N, 2.96%. Found: C, 63.45; H, 7.31; N, 3.21%.

Preparation 7



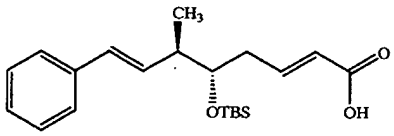
HPLC: C18 reverse phase, flow rate 1 mL/min, 60:40-CH_3CN:H_2O, l=254 nm, a-epoxide Rt=21.0 mins (AUC 1.0); $[a]_D^{589}$ +10.68° (c 1.03, CH_2Cl_2); ^1H NMR ($CDCl_3$) d 7.38–7.26 (m, 6H, ArH_5, 3-H), 6.13 (d, J=15.7 Hz, 2-H), 3.94–3.89 (m, 5-H), 3.60 (s, 8-H), 2.99 (dd, J=7.3 and 1.3 Hz, 7-H), 2.85 (s, 4H, CH_2CH_2), 2.76–2.71 (m, 4-H), 2.61–2.54 (m, 4-H'), 1.64 (dt, J=7.2 and 2.8 Hz, 6-H), 1.03 (d, J=7 Hz, 6-Me), 0.90 (s, 9H, $SiMe_3$), 0.08 (s, SiMe), 0.05 (s, SiMe) ppm; IR ($CHCl_3$) u 2957, 2931, 1741, 1773, 1649, 1254, 1200, 1125, 1095, 1069, 891, 839 cm^{-1}; UV (EtOH) l_{max} 218 (e=21727) nm; MS (FD) m/z 474 (M^+, 10), 416 ([M-CMe_3]$^+$, 100); Anal. calcd. for $C_{25}H_{35}NO_6$ requires: C, 63.40; H, 7.45; N, 2.96%. Found: C, 63.20; H, 7.63; N, 3.07%.

Preparation 8



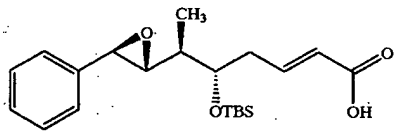
Preparation of β-Epoxy Fragment A Acid

A solution of 2a' (1.91 g, 5.30 mmol) of the formula



in CH_2Cl_2 (18 mL) was treated with m-chloroperbenzoic acid (0.96 g, 5.6 mmol) and the mixture stirred for 4 h before the volatiles were evaporated to give a colorless oil (2.88 g). Preparative HPLC was used to separate the epoxides (1.2:1 β:α) to give the desired β-epoxide as a colorless solid (42%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–7.27 (m, 5H), 7.11 (ddd, 1H, J=15.5, 7.6, 7.6 Hz), 5.92 (d, 1H, J=15.5 Hz), 3.90 (ddd, 1H, J=5.6, 5.6, 5.4 Hz), 3.70 (d, 1H, J=2.0 Hz), 3.00 (dd, 1H, J=6.6, 2.1 Hz), 2.51 (dd, 2H, J=6.5, 6.5 Hz), 1.77–1.73 (m, 1H), 1.10 (d, 3H, J=6.8 Hz), 0.89 (s, 9H), 0.07 (s, 3H), 0.03 (s, 3H). MS (FD) m/z 377 (M+1, 43), 319 (M-57, 100).

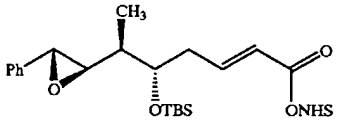
Preparation 9



Alternate Preparation of β-Epoxy Fragment A Acid

To a stirred solution of acid 2a' (100 mg, 0.277 mmol) in CH_3CN (3.7 mL) at 0° C. was added a solution of Na_2EDTA (1×10^{-4} M in H_2O, 2.8 mL, 0.28 μmol) and tetrabutylammonium hydroxide (1 M in MeOH, 28 μL, 28 μmol). After $NaHCO_3$ (23.3 mg, 0.277 mmol) was added, the pH was adjusted to 8.0 with 2 M NaOH and a mixture of Oxone (1.70 g, 2.77 mmol) and $NaHCO_3$ (722 mg, 8.59 mmol) prepared. A 100 mg portion of the Oxone/$NaHCO_3$ was added followed by ketone (7) (143 mg, 0.554 mmol). The pH was immediately adjusted to 7.8–8.0 with 2 M NaOH. The rest of the Oxone/$NaHCO_3$ mixture was added in 95 mg portions in 10 min intervals and a solution of (7) (143 mg, 0.554 mmol) in CH_3CN (500 μL) was added to the mixture during this period via a syringe pump. Throughout the experiment the pH was maintained at 7.8–8.0 with 2 M NaOH and 1 N H_2SO_4. HPLC analysis (C18 reverse phase, detection at 220 nm, flow rate at 1 mL/min, CH_3CN (0.05% TFA)/H_2O (0.05% TFA)-% CH_3CN: 80% to 90% over 10 min) 3 h after the Oxone addition revealed that the conversion was greater than 95% with a β/α epoxide ratio of 5.0:1. The mixture was filtered and the wetcake washed with CH_2Cl_2 (15 mL). The filtrate was washed with H_2O (15 mL) and the aqueous phase back extracted with CH_2Cl_2 (15 mL). The combined organic phases were washed with 0.1 M HCl (10 mL) and H_2O (10 mL), dried ($MgSO_4$), and concentrated to give the crude product as a yellow oil (104 mg, 100%).

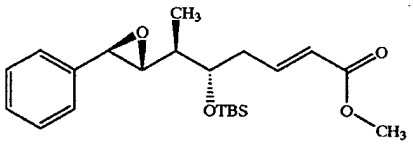
Preparation 10



Alternate Preparation of β-Epoxy Fragment A N-Hydroxysuccinimide Ester

The epoxidation of N-hydroxysuccinimide ester of Preparation 3 (127 mg, 0.277 mmol) was performed in the same manner as described in Preparation 9 except that the pH was lowered to 5.6 with 1 N H_2SO_4 after the tetrabutylammonium hydroxide was added, prior to addition of sodium bicarbonate. HPLC analysis (same method as used for the analysis used in Preparation 9) 3.5 h after the Oxone addition revealed that the conversion was greater than 95% with a β/α epoxide ratio of 6.3:1. After CH_2Cl_2 (6 mL) was added, the mixture was filtered and the wetcake washed with CH_2Cl_2 (14 mL). The filtrate was washed with H_2O (10 mL) and the aqueous phase back extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were dried ($MgSO_4$) and concentrated to a colorless oil. Chromatography on silica gel with EtOAc/hexane (1:3) gave the title compound as colorless solid (8:1 β/α epoxide mixture, 82 mg, 63%).

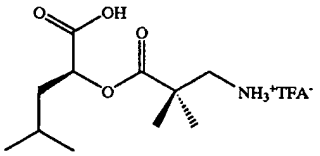
Preparation 11



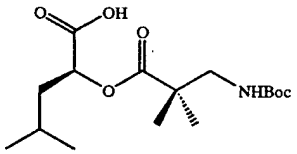
Preparation of β-Epoxy Fragment A Methyl Ester

The epoxidation of the methyl ester of Preparation 1, step 10 (104 mg, 0.278 mmol) was performed in the same manner as described in Preparation 9 except that the pH was lowered to 3.3 with 1 N H_2SO_4 after the tetrabutylammonium hydroxide was added, prior to the addition of sodium bicarbonate. HPLC analysis (same method as used for the analysis of the product of Preparation 9 except % CH_3CN: 95%, isocratic) 2 h after the Oxone addition revealed that conversion was greater than 95% with a β/α epoxide ratio of 4.9:1. After CH_2Cl_2 (6 mL) was added, the mixture was filtered and the wetcake washed with CH_2Cl_2 (14 mL). The filtrate was washed with H_2O (10 mL) and the aqueous phase back extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were dried ($MgSO_4$) and concentrated to give the crude product as a yellow oil (123 mg, 113%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5H), 6.99 (ddd, 1H, J=15.8, 7.6, 7.6 Hz), 5.91 (d, 1H, J=15.8 Hz), 3.87 (ddd, 1H, J=5.6, 5.6, 5.4 Hz), 3.75 (s, 3H), 3.70 (d, 1H, J=2.1 Hz), 3.00 (dd, 1H, J=6.8, 2.1 Hz), 2.49–2.45 (m, 2H), 1.75–1.69 (m, 1H), 1.10 (d, 3H, J=6.8 Hz), 0.88 (s, 9H), 0.06 (s, 3H), 0.02 (s, 3H). MS (FD) m/z 391 (M+1, 8), 333 (M-57, 100).

Preparation 12



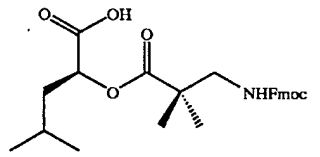
Boc amine (1.69 g,5.09 mmols) of the formula



PCT Intnl. Publ. No. WO 97/07798, published Mar. 6, 1997; was dissolved in trifluoroacetic acid (17 ml) and the solution stirred at room temperature under a dry nitrogen atmosphere for 4.75 h and then concentrated in vacuo and dried under high vacuum for 24 h to give the amine salt as a yellow viscous oil (1.76 g, 100%).

$[\alpha]_D^{589}$ −11.54° (c 1.04, MeOH); ^1H NMR ($CDCl_3$) δ Unit C': 7.43 (br s, 3H, NH_3^+),3.34–3.28 (m, 3-H), 3.18–3.12 (m, 3-H'), 1.42 (s, 2-Me), 1.36 (s, 2-Me); Unit D: 10.94 (br s, CO_2H), 5.23–5.20 (m, 2-H),1.92–1.77 (m, 3H, 3-HH', 4-H), 1.10 (d, J=5.8 Hz, 5-H_3), 0.98 (d, J=5.8 Hz, 4-Me) ppm; IR ($CHCl_3$) ν 2963, 1746, 1710, 1678, 1192, 1172 cm^{-1}; MS (FAB) 232.2 ([M+1]$^+$, 100).

Preparation 13

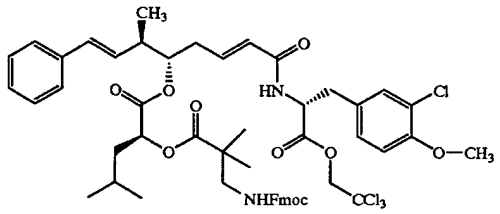


To a stirred solution of amine salt of Preparation 12 (5.09 mmols) in dioxane (20 mL) was added sodium bicarbonate (2.14 g,25.5 mmols) followed by FmocCl (1.58 g,6.11 mmols) at room temperature. The mixture was diluted with H_2O (4 mL) and stirred for 19 h. The reaction mixture was quenched in 1N aqueous HCl (150 mL) and extracted with EtOAc (2×100 mL). Combined organics were washed with H_2O (100 mL), dried ($MgSO_4$) and concentrated in vacuo to give a yellow gummy solid. The crude product was purified by column chromatography (Biotage-SiO_2: gradient elution; 10%–75% EtOAC: Hexanes) to provide Fmoc amine as a pale yellow solid (850 mg, 37%). Product was contaminated with amino acid, which was removed by dissolving the product in EtOAc and stirring with 1N HCl aq for several hours. Organics were dried and concentrated to give product (85:15 product: amino acid).

$[\alpha]_D^{589}$ −15.95° (c 0.50, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ Unit C': 7.59 (d, J=7.4 Hz, ArH_2), 7.67–7.61 (m, ArH_2), 7.43 (t, J=7.3 Hz, ArH_2), 7.36–7.30 (m, ArH_2), 5.88 (t, J=5.8 Hz, NH), 4.41–4.38 (m, 3'-HH'), 4.35–4.28 (m,4'-H), 3.42 (d, J=6.5 Hz, 3-HH'), 1.27 (s, 2Me), 1.26 (s, 2-Me); Unit D: 8.40 (br s, CO2H), 5.18–5.13 (m, 2-H), 1.87–1.69 (m, 3H, 3-HH', 4-H), 0.97 (d, J=5,8 Hz, 5-H3), 0.93 (d, J=6.1 Hz, 4-Me)

ppm; IR (KBr) ν 2959, 2937, 1730, 1540, 1471, 1451, 1307, 1268, 1145, 1128, 759, 741 cm^{-1}; UV (EtOH) λ_{max} 299 (e=5851), 288 (e=4773), 265 (e=18369), 227 (e=4813) nm; MS (FAB) 454 ([M+1]$^+$, 26); Anal. calcd. for $C_{26}H_{31}NO_6$ requires: C, 68.86; H, 6.89; N, 3.09%. Found: C, 68.92; H, 7.01; N, 3.34%.

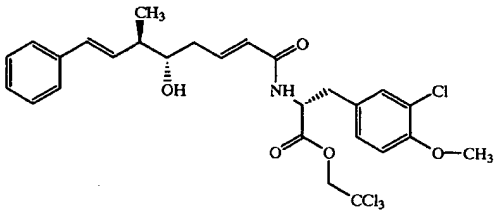
Preparation 14



Preparation of Fmoc Seco

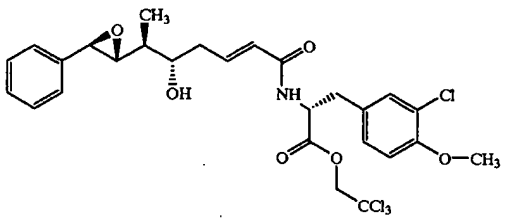
To a mixture of a fragment A-B compound (4')

(4')



(600 mg, 1.01 mmol, Barrow, R. A. et al., *J. Am. Chem. Soc.* 117, 2479–2490 (1995)), a compound of Preparation 13 (618 mg, 1.36 mmol), and DMAP (24.7 mg, 0.202 mmol) in CH_2Cl_2 (3.6 mL) at 0° C. was added a solution of DCC (281 mg, 1.36 mmol) in CH_2Cl_2 (1.2 mL) and the reaction was allowed to stir 5 min at 0° C. and 30 min at rt. After the mixture was diluted with EtOAc/hexane (1:1, 15 mL), it was filtered through Celite and the cake washed with EtOAc/hexane (1:1, 15 mL). The filtrate was washed with 1 M HCl (10 mL), saturated $NaHCO_3$ solution (10 mL), brine (10 mL), dried ($MgSO_4$), and concentrated to a yellow foam. Chromatography on silica gel with EtOAc/hexane (1:2.5 to 1:1) gave the title compound as a colorless foam (864 mg, 83.5%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, 2H, J=7.5 Hz), 7.69–7.66 (m, 2H), 7.40 (dd, 2H, J=7.5, 7.4 Hz), 7.34–7.22 (m, 7H), 7.19 (d, 1H, J=1.8 Hz), 7.05 (dd, 1H, J=8.5, 1.8 Hz), 6.86–6.80 (m, 1H), 6.82 (d, 1H, J=8.4 Hz), 6.45 (d, 1H, J=8.4 Hz), 6.44 (d, 1H, J=15.5 Hz), 6.11–6.03 (m, 2H), 5.91 (d, 1H, J=15.7 Hz), 5.18 (m, 1H), 5.08–5.01 (m, 2H), 4.77 and 4.67 (AB quartet, 2H, J=11.9 Hz), 4.43–4.33 (m, 2H), 4.27 (dd, 1H, J=7.4, 7.2 Hz), 3.86 (s, 3 H), 3.43 (d, 2H, J=6.6 Hz), 3.21 (dd, 1H, J=14.1, 5.7 Hz), 3.07 (dd, 1H, J=14.2, 6.7 Hz), 2.69–2.58 (m, 3H), 1.79–1.72 (m, 2H), 1.67–1.61 (m, 1H), 1.29 (s, 3H), 1.20 (s, 3H), 1.17 (d, 3H, J=6.8 Hz), 0.91 (d, 3H, J=6.4 Hz), 0.87 (d, 3H, J=6.4 Hz). Anal. Calcd for $C_{53}H_{58}Cl_4N_2O_{10}$: C, 62.11; H, 5.70; N, 2.73. Found: C, 62.21; H, 5.68; N, 2.50.

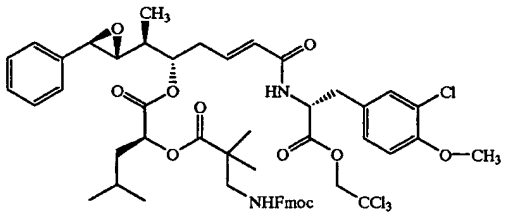
Example 1



Preparation of β-Epoxy Fragment A-B

The epoxidation of fragment A-B of formula (4') (653 mg, 1.11 mmol, Barrow, R. A. et al., *J. Am. Chem. Soc.* 117, 2479–2490 (1995)) was performed in the same manner as described in Procedure 9 except that the pH was lowered to 4.4 with 1 N H_2SO_4 after the tetrabutylammonium hydroxide was added, prior to addition of sodium bicarbonate. The reaction was complete before all of the Oxone/$NaHCO_3$ mixture and ketone were added. HPLC analysis (same method as used for the analysis of the product of Procedure 9 except % CH_3CN: 70% to 85% over 15 min) revealed that the Fragment A-B starting material was consumed to give a 5:1 β/α epoxide mixture after 80% of the Oxone/$NaHCO_3$ mixture had been added (1.6 equivalents of the 2 equivalents of (7) which were added slowly had been added). After CH_2Cl_2 (24 mL) was added, the mixture was filtered and the wetcake washed with CH_2Cl_2 (56 mL). The filtrate was washed with H_2O (40 mL) and the aqueous phase back extracted with CH_2Cl_2 (2×40 mL). The combined organic phases were dried ($MgSO_4$) and concentrated to give the crude product as a light yellow foam (798 mg, 119%), which was used directly in the next reaction without purification. ^1H NMR (500 MHz, $CDCl_3$) δ 7.39–7.29 (m, 5H), 7.21 (d, 1H, J=2.2 Hz), 7.06 (dd, 1H, J=8.4, 2.1 Hz), 6.93 (ddd, 1H, J=15.4, 7.4, 7.4 Hz), 6.88 (d, 1H, J=8.4 Hz), 5.94 (d, 1H, J=15.4 Hz), 5.90 (d, 1H, J=7.8 Hz), 5.09 (ddd, 1H, J=7.7, 6.0, 5.9 Hz), 4.82 and 4.76 (AB quartet, 2H, J=11.8 Hz), 3.90 (s, 3H), 3.83 (d, 1H, J=2.1 Hz), 3.78–3.74 (m, 1H), 3.25 (dd, 1H, J=14.2, 5.9 Hz), 3.15 (dd, 1H, J=14.2, 6.0 Hz), 3.02 (dd, 1H, J=6.7, 2.1 Hz), 2.58–2.52 (br m, 1H), 2.39 (ddd, 1H, J=14.8, 7.6, 7.6 Hz), 1.79 (m, 1H), 1.14 (d, 3H, J=6.9 Hz). MS (FD) m/z 603 (M$^+$).

Example 2

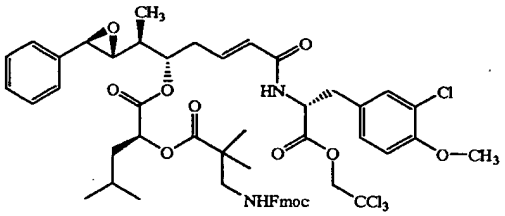


Preparation of β-Epoxy Fmoc Seco from Fmoc Seco

To a stirred solution of a compound of Preparation 14 (382 mg, 0.373 mmol) in CH_3CN (4.7 mL) at 0° C. was added a solution of Na_2EDTA (1×10^{-4} M in H_2O, 3.7 mL, 0.37 μmol) and tetrabutylammonium hydroxide (1 M in MeOH, 37 μL, 37 μmol). The pH was lowered to 3–6 with 1 N H_2SO_4 before $NaHCO_3$ (31.3 mg, 0.373 mmol) was added. After a mixture of Oxone (2.29 g, 3.72 mmol) and $NaHCO_3$ (975 mg, 11.6 mmol) was prepared, the pH was adjusted to 8.2 with 1 N H_2SO_4 and the ketone (7) (385 mg,

1.49 mmol) added. The Oxone/$NaHCO_3$ mixture was then added in 128 mg portions in 10 min intervals and the pH maintained at 7.8–8.2 with 2 M NaOH and 1 N H_2SO_4. HPLC analysis (same method as used for the analysis of the product of Procedure 9 except % CH_3CN: 95%, isocratic) 1 h after the Oxone addition revealed that the conversion was 85% with a β/α epoxide ratio of 9.5:1. After CH_2Cl_2 (8 mL) was added, the mixture was filtered and the wetcake washed with CH_2Cl_2 (19 mL). The filtrate was washed with H_2O (13 mL) and the aqueous phase back extracted with CH_2Cl_2 (2×27 mL). The combined organic phases were dried ($MgSO_4$) and concentrated to give the crude product as a beige foam (375 mg, 97%).

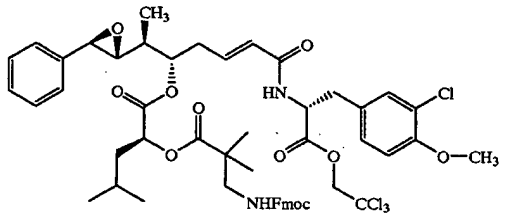
Example 3



Preparation of β-Epoxy Fmoc Seco from β-Epoxy Fragment A-B

A solution of the epoxide product of Example 1 (788 mg, 1.09 mmol theory) in CH_2Cl_2 (3.7 mL) was added to the acid of Preparation 13 (742 mg, 1.64 mmol) followed by DMAP (26.6 mg, 0.218 mmol), and the mixture was immediately cooled to 0° C. After a solution of DCC (338 mg, 1.64 mmol) in CH_2Cl_2 (1.2 mL) was added, the mixture was allowed to stir for 5 min at 0° C. and for 1 h at rt. The mixture was then diluted with EtOAc/hexane (1:1, 20 mL), filtered through Celite, and concentrated to a yellow foam. Chromatography on silica gel with EtOAc/hexane (1:3 to 1:2) gave the title compound as a light yellow foam (7.8:1 β/α epoxide mixture, 772 mg, 68% from Fragment A-B corrected to 55% due to contamination by byproduct). ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, 2H, J=7.5 Hz), 7.66–7.60 (m, 2H), 7.40–7.24 (m, 9H), 7.19 (s, 1H), 7.05 (d, 1H, 8.4 Hz), 6.86–6.79 (m, 1H), 6.82 (d, 1H, J=8.4 Hz), 6.44 (d, 1H, J=8.0 Hz), 5.98–5.94 (br m, 1 H), 5.91 (d, 1H, J=15.6 Hz), 5.23–5.20 (m, 1H), 5.09–5.04 (m, 1H), 5.0 (dd, 1H, J=9.8, 3.2 Hz), 4.77 and 4.68 (AB quartet, 2H, J=11.8 Hz), 4.43–4.34 (m, 2H), 4.27–4.23 (m, 1H), 3.85 (s, 3H), 3.73 (d, 1H, J=1.7 Hz), 3.44–3.38 (m, 2H), 3.20 (dd, 1H, J=14.2, 5.7 Hz), 3.07 (dd, 1H, J=14.2, 6.7 Hz), 2.94 (dd, 1H, J=6.9, 1.7 Hz), 2.66–2.57 (m, 2H), 1.97–1.90 (m, 1H), 1.80–1.71 (m, 2H), 1.60–1.55 (m, 1H), 1.25 (s, 3H), 1.19 (s, 3H), 1.15 (d, 3H, J=6.9 Hz), 0.93 (d, 6H, J=6.4 Hz), MS (FD) m/z 1038 (M^+).

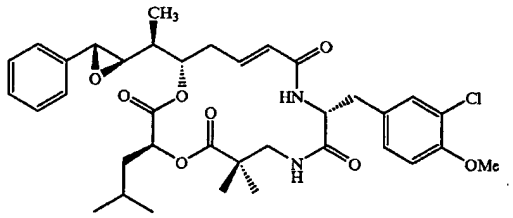
Example 4



Preparation of β-Epoxy Fmoc Seco from Fragment A-B: Epoxidation at pH 10.5 followed by coupling with preactivation of Fmoc C'D Acid

Epoxidation: To a stirred mixture of fragment A-B (4') (650 mg, 1.10 mmol), sodium tetraborate buffer (0.05 M in 4×10^{-4} M aqueous Na_2EDTA, 11.2 mL), tetrabutylammonium hydrogen sulfate (14.9 mg, 43.9 μmol), and ketone (7) (568 mg, 2.20 mmol) in CH_3CN (16.8 mL) at 0° C. was added a solution of Oxone (2.61 g, 4.24 mol) in aqueous Na_2EDTA (4×10^{-4} M, 20 mL) and a solution of K_2CO_3 (0.89 M, 20 mL) over 2 h. HPLC analysis (same method as used for the analysis of the product of Preparation 9 except % CH_3CN: 70% to 85% over 15 min) at the end of the addition revealed that the conversion was >95% with a β/α epoxide ratio of 6.5:1. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (20 mL). The aqueous layer was back extracted with CH_2Cl_2 (2×30 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated to faint yellow foam (995 mg) which was taken into the next step without further purification. Coupling: To a solution of the acid (10a) (723 mg, 1.60 mmol) and DMAP (26.9 mg, 0.220 mmol) in CH_2Cl_2 (1.7 mL) at 0° C. was add a solution of the DCC in CH_2Cl_2 (1.3 mL). After the mixture was stirred for 5 min, a solution of the crude alcohol in CH_2Cl_2 (1.5 mL) was added and the reaction was stirred for 5 min at 0° C. and 1 h at rt. The mixture was then diluted with EtOAc/hexane (1:1, 20 mL), filtered through Celite, and concentrated to a yellow foam. Chromatography on silica gel with EtOAc/hexane (1:3 to 1:2) gave the title compound as a light yellow foam (10.3:1 β/α epoxide mixture, 820 mg, 71% contaminated with only 4% of byproduct).

Example 5

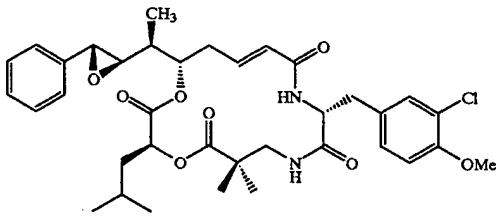


Preparation of Cryptophycin 52 from Cryptophycin 51

The epoxidation of Cryptophycin 51 (181 mg, 0.277 mmol) was performed in the same manner as described in

Preparation 9 except that the pH was lowered to 7.8 with 1 N H_2SO_4 after the tetrabutylammonium hydroxide was added, prior to addition of sodium bicarbonate. HPLC analysis (same method as used for the analysis of the product of Preparation 9 except % CH_3CN: 60% to 90% over 20 min) near the end of the Oxone addition revealed that the conversion was less than 10% with a β/α epoxide ratio of 5–7:1.

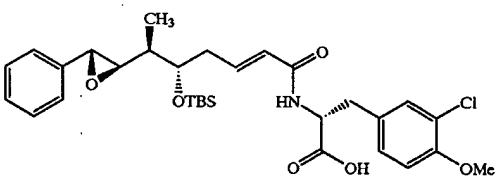
Example 6



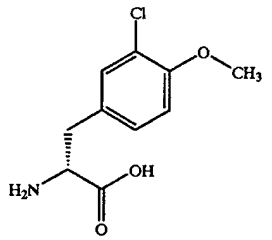
Preparation of Cryptophycin 52 from β-Epoxy Fmoc Seco

To a solution of β-Epoxy Fmoc Seco of Example 3 (767 mg, 0.737 mmol corrected to 0.54 mmol due to contamination by α-epoxide and byproduct) in DMF (74 mL) was added piperidine (364 μL, 3.69 mmol), and the reaction was allow to stir for 20 h before it was diluted with EtOAc (200 mL) and washed with H_2O (3×200 mL). The combined aqueous layers were back extracted with EtOAc (60 mL) and the combined organic layers dried ($MgSO_4$) and concentrated to an orange oil. Crystallization from toluene (2.5 mL, seeded with Cryptophycin 52) provided the title compound as a colorless solid (171 mg, 47%).

Example 7



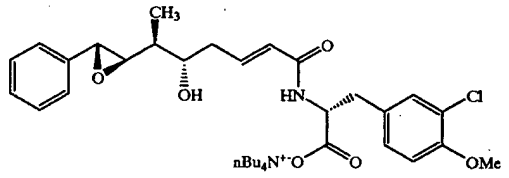
To a solution of β-epoxide of Preparation 6 (473 mg, 1.0 mmol) in dry DMF (6.7 mL) was added amino acid "B" (459 mg, 2.0 mmols), represented by the formula



PCT Intnl. Publ. No. WO 97/07798, published Mar. 6, 1997; followed by N,O-bis-(trimethylsilyl)acetamide (618 uL, 2.5 mmols) at room temperature under a nitrogen atmosphere. The resulting mixture was heated at 55° C. (solution formed) for 8 h, diluted with EtOAc (250 mL) and washed with 1N aqueous HCl (3×80 mL), H_2O (100 mL). Combined, dried ($MgSO_4$) organics were concentrated in vacuo to give a yellow foam (590 mg), which further purified by column chromatography (SiO_2, gradient elution; CH_2Cl_2-5%–10% MeOH: CH_2Cl_2) to give silyl ether product as white foam (489 mg, 89%).

$[\alpha]_D^{589}$ +28.33° (c 1.06, MeOH); ^1H NMR (DMSO-d6) δ Unit A: 7.33–7.17 (m, ArH_5), 6.55–6.40 (m, 3-H), 6.03 (d, J=15.3 Hz, 2-H), 3.83–3.76 (m, 5-H), 3.71 (s, 8-H), 2.90 (d, J=6.8 Hz, 7-H), 2.46–2.27 (m, 4-HH'), 1.50–1.44 (m, 6-H), 0.94 (d, J=6.7 Hz, 6-Me), 0.74 (s, 9H, $SiMe_3$), –0.54 (s, SiMe), –0.13 (s, SiMe); Unit B: 7.76 (d, J=7.3, NH), 7.33–7.17 m, ArH), 7.04 (d, J=8.5, ArH), 6.90 (d, J=8.5, ArH), 4.27–4.23 (m, 2-H), 3.72 (s, 3H, OMe), 3.02 (dd, J=13.3 and 4.3 Hz, 3-H), 2.78 (dd, J=13.5 and 7.8 Hz, 3-H') ppm; IR (KBr) u 2955, 2930, 2857, 1668, 1605, 1504, 1463, 1454, 1279, 1258, 1067, 1026, 837, 776 cm^{-1}; UV (EtOH) λ_{max} 278 (e=2219) nm.

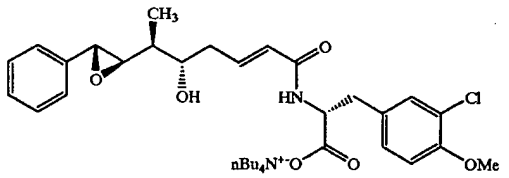
Example 8



Method A

To a solution of silyl ether of Example 7 (160 mg, 0.272 mmols) in dry DMF (3.5mL) was added sodium bicarbonate (228 mg, 2.72 mmols) followed by solid tetrabutylammonium fluoride-hydrate (TBAF)(358 mg, 1.36 mmols). The mixture was heated at 60° C. for 17 h and then further TBAF (358 mg, 1.36 mmols) and heated for 9 h and finally a solution of 1M TBAF in THF (360 uL, 1.36 mmols) added turning the reaction a brown color. The mixture was heated for 20 mins and then the reaction quenched in water (100 mL) and extracted with EtOAc (3×50 mL). Combined, dried (Na_2SO_4) organics were concentrated in vacuo to give a brown oily gum (248 mg). Crude carboxylate salt was used in the next step without further purification.

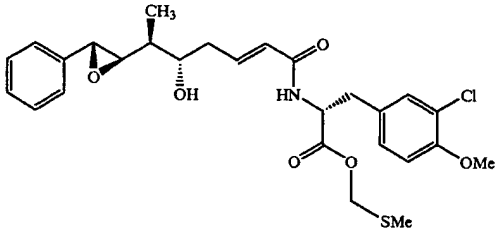
Example 9



Method B

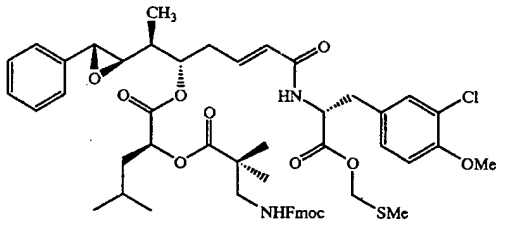
To a solution of silyl ether of Example 7 (145 mg, 0.247 mmols) in dry tetrahydrofuran (3.0 mL) was added a 1M solution of tetrabutylammonium fluoride (800 uL, 0.8 mmols) under a dry nitrogen atmosphere. The resulting solution was heated at 60° C. for 7 h and then worked-up as described above to give a brown residue (166 mg, 94%). Crude carboxylate salt was used in the next step without further purification.

Example 10



To a dry solution of crude carboxylate salt (0.272 mmols) in DMSO (3.5 mL) was sodium bicarbonate (274 mg, 3.26 mmols) followed by slow addition of a solution of t-butyl bromide (373 mg, 2.72 mmols) in DMSO (1.5 mL) over ~2 h at room temperature and under nitrogen. The mixture was stirred for a further 21 h and then quenched in brine (50 mL) and extracted with EtOAc (3x30 mL). Combined organics were washed with water (50 mL), dried (Na_2SO_4) and concentrated in vacuo to give crude ester as a gummy solid (117 mg, 81%). The crude alcohol A-B was used in the next step without further purification.

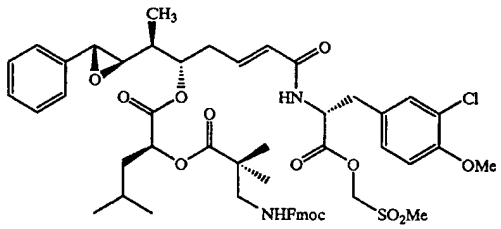
Example 11



To a stirred solution of carboxylic acid D-C' of Preparation 13 (129 mg, 0.285 mmols) in dry dichloromethane (1.0 mL) was added DMAP (5.4 mg, 0.044 mmols) and DCC (59 mg, 0.285 mmols) at room temperature under a dry nitrogen atmosphere. The solution was stirred for 0.5 h and then solid sodium bicarbonate (37 mg, 0.44 mmols) added followed by a solution of crude alcohol A-B of Example 10 (117 mg, 0.22 mmols) in dry dichloromethane (1.2 mL). A precipitate formed within 10 mins and the mixture was stirred for a further 50 mins. The crude reaction mixture was directly applied onto a SiO_2 column and purified (gradient elution; 10%–40% EtOAc:Hexanes) to give methyl sulphide product as pale yellow solid (122 mg, 46% over 3 steps).

^{1}H NMR ($CDCl_3$) δ Unit A: 7.43–7.20 (m, ArH_5), 6.90–6.81 (m, 2H, 3-H, ArH), 5.93 (d, J=15.6 Hz, 2-H), 5.14–4.93 (m, 5-H), 3.05 (dd, J=14.5 and 8.3 Hz, 7-H), 2.65–2.63 (m, 4-HH'), 2.00–1.95 (m, 6-H), 1.17 (d, J=7.0, 6-Me); Unit B: 7.43–7.20 (m, ArH), 7.06 (d, J=8.1 Hz, ArH), 6.90–6.81 (m, ArH), 6.44 (d, J=7.7 Hz, NH), 5.19 (q, J_{AB}=11.8 Hz, 1'-HH), 5.14–4.93 (m, 2-H), 3.87 (s, OMe), 3.20–3.10 (m, 3-HH'), 2.21 (s, SMe); Unit C': 7.79 (d, J=7.4 Hz, ArH_2), 7.67 (d, J=6.9 Hz, ArH_2), 7.43–7.20 (m, ArH_4), 6.04 (d, J=7.7 Hz, NH), 4.42–4.34 (m, 3'-HH'), 4.30–4.25 (m, 4'-H, 3.42 (d, J=6.2 Hz, 3-HH'), 1.27 (s, 2-Me), 1.20 (s, 2-Me); Unit D: 5.22–5.18 (m, 2-H), 1.82–1.58 (m, 3H, 3-HH',4-H), 0.96 (s, 5-H3), 0.94 (s, 4-Me) ppm.

Example 12

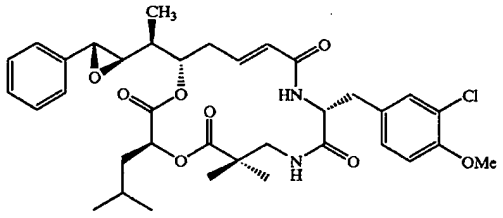


To a stirred solution of methyl sulphide of Example 11 (56 mg,0.058 mmols) in acetone (10 mL) was added sodium bicarbonate (64 mg, 0.764 mmols) followed by an aqueous solution of oxone (234 mg, 0.382 mmols) in water (3.0 mL). The reaction mixture was stirred at room temperature for 20 mins (SM is rapidly converted to a very polar component sulphoxide and then with time to the less polar sulphone product). The reaction was quenched in water (40 mL) and extracted with EtOAc (3x20 mL). Organics were washed with brine (30 mL), dried ($MgSO_4$) and concentrated in vacuo to give a solid. Crude product was purified by column chromatography (SiO_2: gradient elution; 25%–60% EtOAc:Hexanes) to give sulphone as a white foamy solid (43 mg, 74%).

^{1}H NMR ($CDCl_3$) δ Unit A: 7.58–7.17 (m, ArH_5), 6.82–6.75 (m, 3-H), 5.87 (d, J=16 Hz, 2-H), 4.98–4.86 (m, 5-H), 3.70 (d, J=1.1 Hz, 7-H), 2.92–2.89 (m,7-H), 2.61–2.58 (m,4-HH'), 1.94–1.89 (m, 6-H), 1.13 (d, J=7.1 Hz, 6-Me); Unit B: 7.58–7.17 (m, ArH), 7.04 (d, J=7.7 Hz, ArH), 6.81 (d, J=8.1 Hz, ArH), 6.54 (d, J=7.5 Hz, NH), 4.98–4.86 (m, 2-H), 3.84 (s, 7-OMe), 3.17–2.98 (dq, J_{AB}=14 and 6.6 Hz, 2-HH'); Unit C': 7.75 (d, J=7.4 Hz, ArH_2), 7.62 (d, J=6.8 Hz, ArH_2), 7.58–7.17 (m, ArH_4), 5.97 (t, J=5.5 Hz, NH), 5.00 (s, SO_2Me), 4.98–4.86 (m, 2H, 1'-HH'), 4.38–4.33 (m, 3'-HH'), 4.25–4.20 (m, 4'-H), 3.40–3.36 (m, 3-HH'), 1.22 (s, 2-Me), 1.15 (s, 2-Me); Unit D: 5.19 (q, J_{AB}=5 Hz, 2-H), 1.80–1.61 (m, 2H, 3-H, 4-H), 1.57–1.49 (m, 3-H'), 0.91 (s, 5-H_3), 0.89 (s, 4-Me) ppm.

Example 13

Cryptophycin 52



To a stirred solution of sulphone of Example 12 (18 mg, 17.98 umols) in dry DMF (2.0 mL) was added neat piperidine (8.9 uL, 90 umols) at room temperature and under nitrogen. The resulting solution was stirred for 5 h and then concentrated in vacuo to give crude amine as a foam. The amine was dissolved in toluene (3 mL) and heated at 60° C. under nitrogen for 40 mins. The reaction solution was directly purified by column chromatography (SiO_2; gradient elution; 20%–75% EtOAc:Hexanes) to give cryptophycin 52 as a white glass (6.1 mg, 51% over 2 steps).

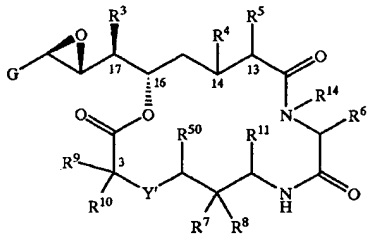
^{1}H NMR ($CDCl_3$) δ Unit A: 7.45–7.38 (m, ArH_3), 7.31–7.23 (m, ArH_2), 6.85–6.76 (m, 3-H), 5.76 (d, J=15.6 Hz, 2-H), 5.27–5.23 (m, 5-H), 2.97 (dd, J=7.5 and 1.7 Hz, 7-H), 2.66–2.44 (m, 4-HH'), 1.86–1.67 (m, 6-H), 1.19 (d, J=6.9 Hz, 6-Me); Unit B: 7.31–7.23 (m, ArH), 7.09 (dd, J=8.3 and 2.0 Hz, ArH), 6.88 (d, J=8.4 Hz, ArH), 5.50 (d J=7.8 Hz, NH), 4.79 (q, J=6.4 Hz, 2-H), 3.92 (s, OMe), 3.73 (d, J=1.5 Hz, 8-H), 3.17–3.11 (m, 3-HH'); Unit C': 3.47 (dd, J=13.4 and 8.7 Hz, 3-H), 3.17–3.11 (m, 3'-H), 1.27 (s, 2-Me), 1.20 (s, 2-Me); Unit D: 4.87 (dd, J=10 and 3.3 Hz, 2-H), 1.86–1.67 (m, 2H, 3-H, 4-H), 1.40–1.30 (m, 3-H'), 0.88 (app t, J=6.3 Hz, 6H, 5-H_3, 4-Me) ppm.

Conversion rates and β/α ratios are presented in Table 1 below. Epoxidations were completed in CH_3CN/aq. H_2O at 0° C. and pH 7.8–8.2 using 4 equivalents of (7) (2 of the 4 equivalents added over 4 hours) and a mixture of Oxone (10 equivalents) and $NaHCO_3$ (31 equivalents) which was added over 4 hours. For entry 2, all of the ketone (7) was added at the beginning.

We claim:

1. A process for preparing a compound of the formula

(I)



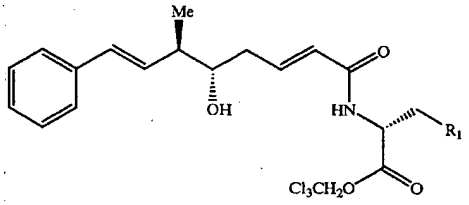
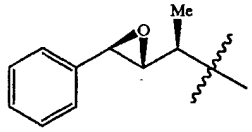
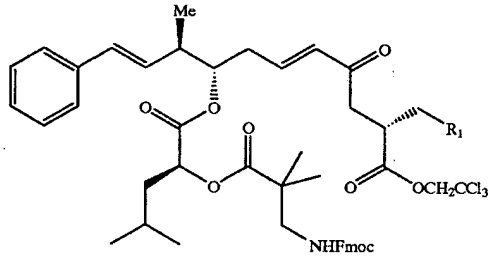
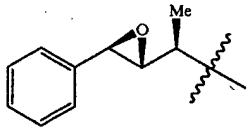
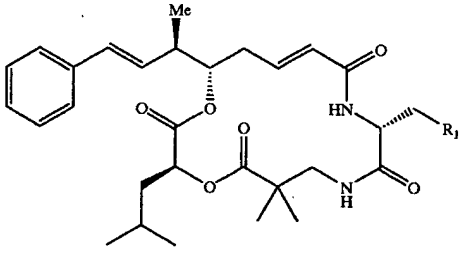
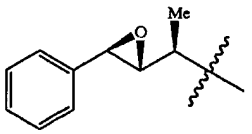
wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

TABLE 1

Asymmetric Epoxidation of Cryptophycin Intermediates

No.	Substrate	Product	β/α ratio	Conversion
1	 R_1 = 3-Cl-4-methyoxyphenyl	 Example 1	5:1	>95%
2	 R_1 = 3-Cl-4-methyoxyphenyl	 Example 2	9.5:1	85%
3	 R_1 = 3-Cl-4-methyoxyphenyl	 Example 5	5–7:1	<10%

R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^7 and R^8 are each independently hydrogen or C_1–C_6 alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, C_1–C_6 alkyl, C_2–C_6 alkenyl, C_2–C_6 alkynyl, —$(CH_2)_m$—$(C_3$–$C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or C_1–C_6 alkyl;

R^{11} is hydrogen, C_1–C_6 alkyl, phenyl or benzyl;

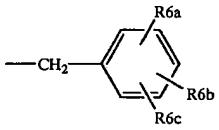
R^{14} is hydrogen or C_1–C_6 alkyl;

R^{50} is hydrogen or (=O);

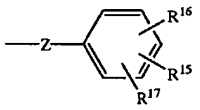
Y is CH, O, NH, SO, SO_2 or $(C_1$–$C_3)$alkylamino;

R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

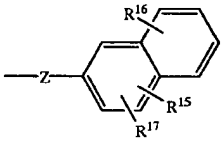
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, halo or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1$–$C_6)$ alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, $(C_1$–C_6alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or C_1–C_6 alkyl;

R^{23} is hydrogen or $(C_1$–$C_3)$alkyl;

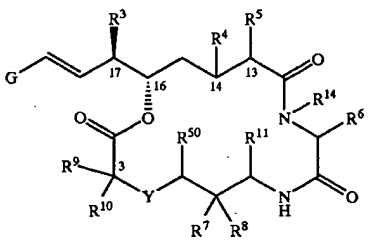
Z is —$(CH_2)_{11}$— or $(C_3$–$C_5)$cycloalkyl;

n is 0, 1, or 2; and

Z' is an aromatic or substituted aromatic group; or a pharmaceutically acceptable salt thereof;

comprising epoxidizing a compound of the formula

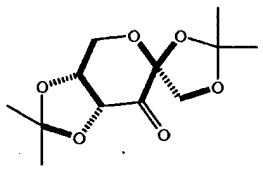
(4)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14} and R^{50} are as defined above and Y is Y' or S; with an oxidant and a chiral ketone to form a compound of formula (I); and optionally forming a pharmaceutically acceptable salt thereof.

2. A process according to claim **1** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with —$CH_2OC(O)(CH_2)_m NH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; R^6 is a group of the formula (IA).

3. A process according to claim **1** wherein said oxidant is Oxone and said chiral ketone is a compound of the formula

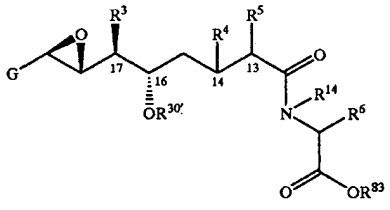


4. A process according to claim **2** wherein G is phenyl.

5. A process according to claim **1** wherein said compound of formula (I) is Cryptophycin 52.

6. A process for preparing a compound of the formula

(II)



wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

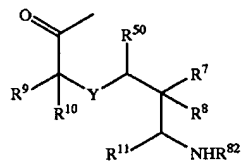
Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^{83} is hydrogen, C_1–C_6 alkyl, trichloroethyl, or —CH_2SR^{81};

$R^{30'}$ is hydrogen, an alcohol protecting group, or a group of the formula



R^7 and R^8 are each independently hydrogen or C_1–C_6 alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, C_1–C_6 alkyl, C_2–C_6 alkenyl, C_2–C_6 alkynyl, —$(CH_2)_m$—$(C_3$–$C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or C_1–C_6 alkyl;

R^{11} is hydrogen, C_1–C_6 alkyl, phenyl or benzyl;

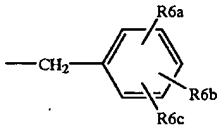
R^{14} is hydrogen or C_1–C_6 alkyl;

R^{50} is hydrogen or (=O);

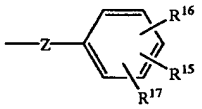
Y is CH, O, NR^{12}, S, SO, SO_2, wherein R^{12} is H or C_1–C_3 alkyl;

R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

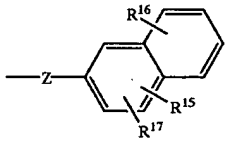
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, $(C_1$–$C_6)$alkyl, halo $NR^{18}R^{19}$ or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1$–$C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, $(C_1$–C_6 alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or C_1–C_6 alkyl;

R^{23} is hydrogen or $(C_1$–$C_3)$alkyl;

Z is —$(CH_2)_n$— or $(C_3$–$C_5)$cycloalkyl;

n is 0, 1, or 2; and

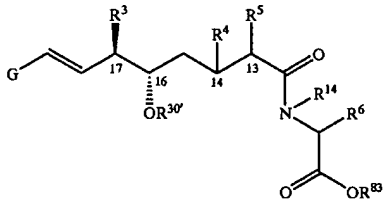
Z' is an aromatic or substituted aromatic group;

R^{81} is C_1–C_6 alkyl, C_3–C_8 cycloalkyl, phenyl or benzyl; and

R^{82} is a base labile protecting group; or a pharmaceutically acceptable salt thereof; with the proviso that when R^{83} is —CH_2SR^{81}, R^{30} is not hydrogen or an alcohol protecting group;

comprising epoxidizing a compound of the formula

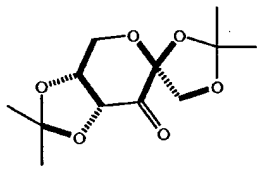
(5)



wherein G, R^3, R^4, R^5, R^6, R^{14}, $R^{30'}$ and R^{83} are as defined above; with an oxidant and a chiral ketone to form a compound of formula (II); and optionally forming a pharmaceutically acceptable salt thereof.

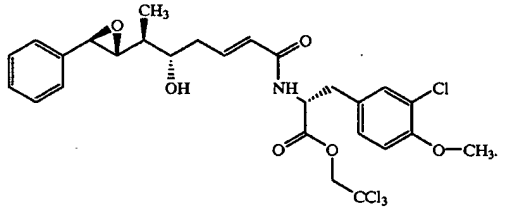
7. A process according to claim **6** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with —$CH_2OC(O)(CH_2)_m$-NH_2; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; R^6 is a group of the formula (IA), $R^{30'}$ is hydrogen.

8. A process according to claim **6** wherein said oxidant is Oxone and said chiral ketone is a compound of the formula



9. A process according to claim **7** wherein G is phenyl and R^{83} is trichloroethyl.

10. A process according to claim **6** wherein said compound of formula (II) is represented by the formula

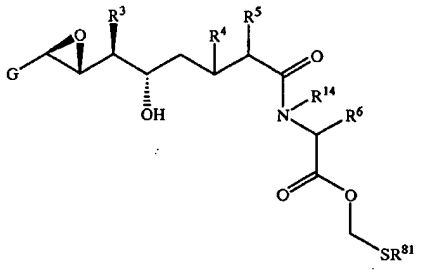


11. A process according to claim **6** further comprising forming a compound of formula (I).

12. A process for preparing a compound of formula (I) according to claim **6**, further comprising the steps of:

(a) contacting the compound of formula (II), wherein $R^{30'}$ is hydrogen and R^{83} is a cation, with a thioester forming agent to form a compound of the formula

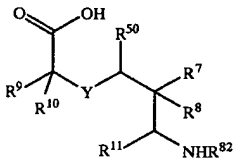
(IIc)



wherein G, R^3, R^4, R^5, R^6 and R^{14} are as defined above and R^{81} is C_1–C_6 alkyl, C_3–C_8 cycloalkyl, phenyl or benzyl;

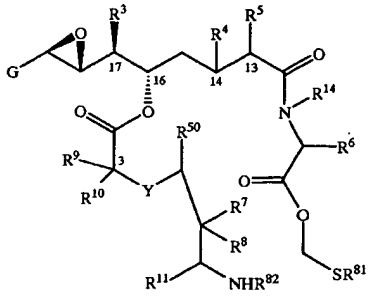
(b) coupling a compound of formula (IIc) with a compound of the formula

(10a)



wherein R^7, R^8, R^9, R^{10}, R^{11} and R^{50} are as defined above and R^{82} is a base labile protecting group, to form a compound of the formula

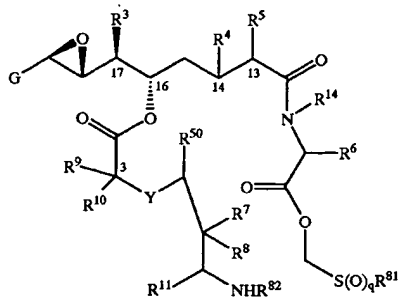
(8)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50} R^{81}, R^{82} and Y are as defined above;

(c) oxidizing a compound of formula (8) with an oxidizing agent to form a compound of the formula

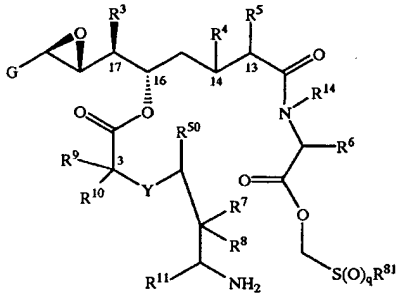
(9)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, R^{81} and R^{82} and Y are as defined above and q is an integer 1 or 2;

(d) deprotecting a compound of formula (9) with a suitable deprotecting agent to form a compound of the formula

(13)



wherein G, R^3, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, Y, q and R^{81} are as defined above; and optionally contacting a compound of formula (10) with a cyclizing agent to form a compound of formula (I); and

(e) optionally forming a pharmaceutically acceptable salt of a compound of formula (I).

13. A process according to claim **12** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with —$CH_2OC(O)(CH_2)_mNH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA); said deprotecting agent is piperidine; said oxidizing agent is Oxone; said oxidant is Oxone and said chiral ketone is a compound of formula (7) and the pH of the epoxidation step is maintained in the range of from about 7.0 to about 11.5.

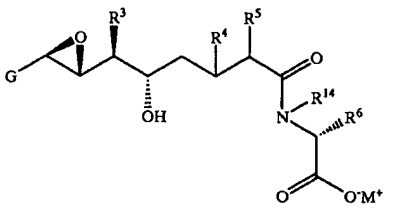
14. A process according to claim **12** wherein said compound of formula (I) is Cryptophycin 52.

15. A process for preparing a compound of formula (I) according to claim **6**, further comprising the steps of:

(a) deprotecting a compound formula (II) wherein $R^{30'}$ is an alcohol protecting group, with a suitable alkoxy deprotecting agent and further carboxy-deprotecting the compound of formula (II) when R^{83} is C_1–C_6 is

alkyl, with a suitable base to form a compound of the formula

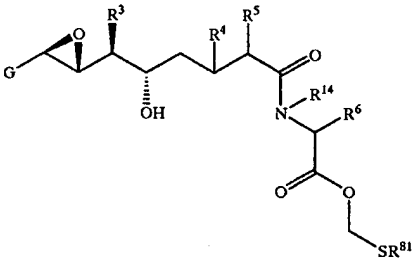
(IIb)



wherein G, R^3, R^4, R^5, R^6 and R^{14} are as defined above and M^+ is a cation;

(b) contacting the compound of formula (IIb), with a thioester forming agent to form a compound of the formula

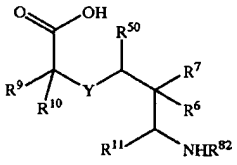
(IIc)



wherein G, R^3, R^4, R^5, R^6 and R^{14} are as defined above and R^{81} is C_1–C_6 alkyl, C_3–C_8 cycloalkyl, phenyl or benzyl;

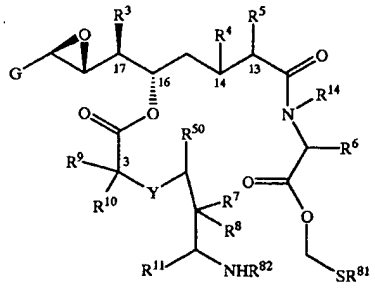
(c) coupling a compound of formula (IIc) with a compound of the formula

(10a)



wherein R^7, R^8, R^9, R^{10}, R^{11} and R^{50} are as defined above and R^{82} is a base labile protecting group, to form a compound of the formula

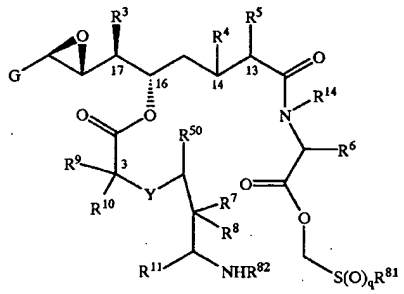
(8)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50} R^{81}, R^{82} and Y are as defined above;

(d) oxidizing a compound of formula (8) with an oxidizing agent to form a compound of the formula

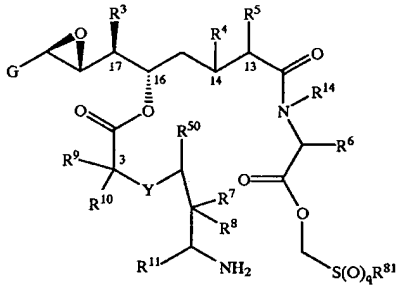
(9)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, R^{81} and R^{82} and Y are as defined above and q is an integer 1 or 2;

(e) deprotecting a compound of formula (9) with a suitable deprotecting agent to form a compound of the formula

(10)



wherein G, R^3, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, Y, q and R^{81} are as defined above; and optionally contacting a compound of formula (10) with a cyclizing agent to form a compound of formula (I); and

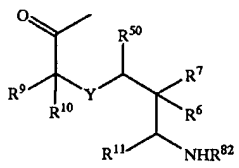
(f) optionally forming a pharmaceutically acceptable salt of a compound of formula (I).

16. A process according to claim **15** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with —$CH_2OC(O)(CH_2)_m.NH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA); said deprotecting agent is piperidine; said oxidizing agent is Oxone; said oxidant is Oxone and said chiral ketone is a compound of formula (7).

17. A process according to claim **15** wherein said compound of formula (I) is Cryptophycin 52.

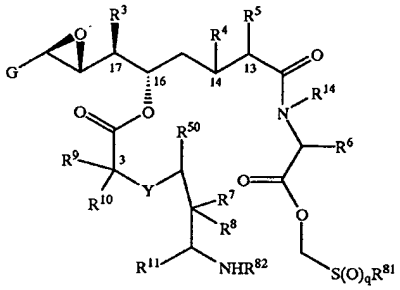
18. A process for preparing a compound of formula (I) according to claim **6** further comprising the steps of:

(a) oxidizing a compound of formula (II) wherein $R^{30'}$ is a compound of the formula



and R^{83} is $—CH_2SR^{81}$, with an oxidizing agent to form a compound of the formula

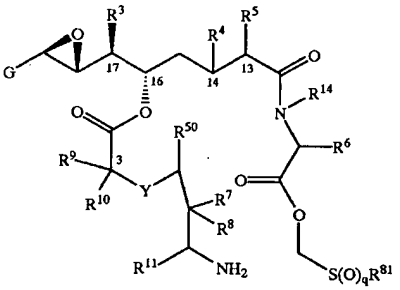
(9)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, R^{81} and R^{82} and Y are as defined above and q is an integer 1 or 2;

(b) deprotecting a compound of formula (9) with a suitable deprotecting agent to form a compound of the formula

(10)



wherein G, R^3, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, Y, q and R^{81} are as defined above; and optionally contacting a compound of formula (10) with a cyclizing agent to form a compound of formula (I); and

(c) optionally forming a pharmaceutically acceptable salt of a compound of formula (I).

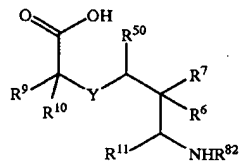
19. A process according to claim **18** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_mNH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA); said deprotecting agent is piperidine; said oxidizing agent is Oxone; said oxidant is Oxone and said chiral ketone is a compound of formula (7).

20. A process according to claim **18** wherein said compound of formula (I) is Cryptophycin 52.

21. A process for preparing a compound of formula (I) according to claim **6**, further comprising the steps of

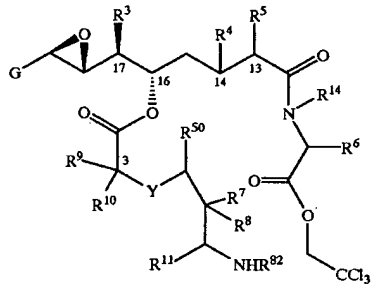
(a) coupling a compound of formula (II), wherein $R^{30'}$ is hydrogen and R^{83} is trichloroethyl, with a compound of the formula

(10a)



wherein R^7, R^8, R^9, R^{10}, R^{11} and R^{50} are as defined above and R^{82} is a base labile protecting group, to form a compound of the formula

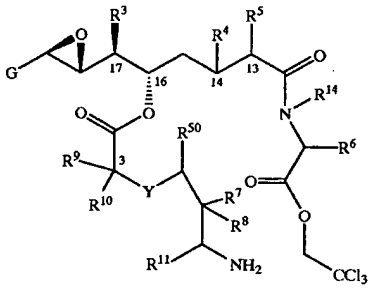
(18)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, R^{82} and Y are as defined above;

(b) treating a compound of formula (18) with a suitable base-deprotecting agent to provide a compound of the formula

(19)



wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50} and Y are as defined above;

(c) cyclizing a compound of formula (19) with a suitable ring-closing agent to provide a compound of formula (I); and

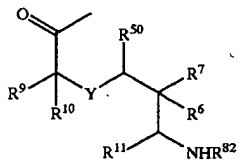
(d) optionally forming a pharmaceutically acceptable salt of a compound of formula (I).

22. A process according to claim **21** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_mNH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA); said oxidizing agent is Oxone; said oxidant is Oxone and said chiral ketone is a compound of formula (7).

23. A process according to claim 21 wherein said compound of formula (I) is Cryptophycin 52.

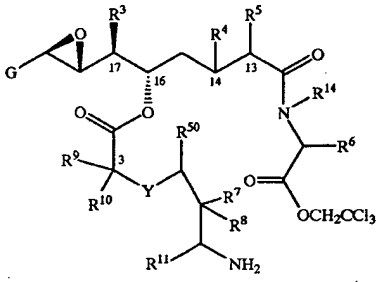
24. A process for preparing a compound of formula (I) according to claim 6, further comprising the steps of

(a) deprotecting a compound of formula (II) wherein $R^{30'}$ is a compound of the formula



and R^{83} is trichloroethyl, with a suitable base-deprotecting agent to form a compound of the formula

(19)



wherein G, R^3, R^4, R^5, R^6 and R^{14} are as defined above;

(b) cyclizing a compound of formula (19) with a suitable ring-closing agent to provide a compound of formula (I); and

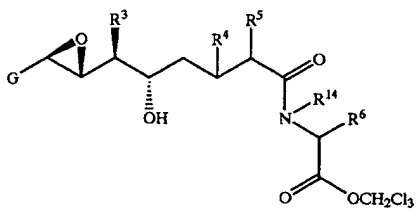
(c) optionally forming a pharmaceutically acceptable salt of a compound of formula (I).

25. A process according to claim 24 wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_m NH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA); said oxidizing agent is Oxone; said oxidant is Oxone and said chiral ketone is a compound of formula (7).

26. A process according to claim 24 wherein said compound of formula (I) is Cryptophycin 52.

27. A compound of the formula

(IId)



wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

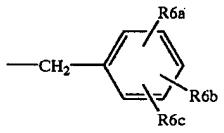
R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

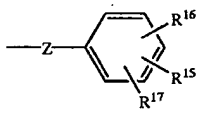
R^{14} is hydrogen or C_1–C_6 alkyl;

R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

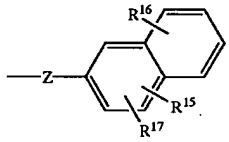
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, halo or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1$–$C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, $(C_1$–C_6 alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or C_1–C_6 alkyl;

R^{23} is hydrogen or $(C_1$–$C_3)$alkyl;

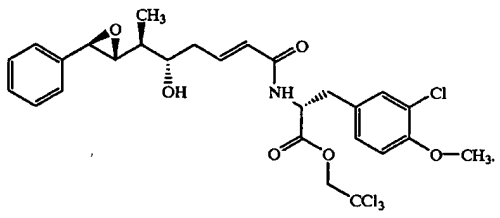
Z is $—(CH_2)_n—$ or $(C_3$–$C_5)$cycloalkyl;

n is 0, 1, or 2; and

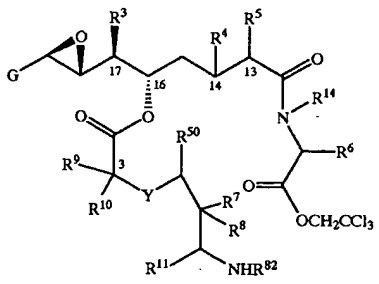
Z' is an aromatic or substituted aromatic group; or a pharmaceutically acceptable salt thereof.

28. A compound according to claim 27 wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_m NH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^{14} is hydrogen; and R^6 is a group of the formula (IA).

29. A compound according to claim 27 wherein said compound is represented by the formula



30. A compound of the formula



(18)

wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^7 and R^8 are each independently hydrogen or C_1–C_6 alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, C_1–C_6 alkyl, C_2–C_6 alkenyl, C_2–C_6 alkynyl, —$(CH_2)_m$—$(C_3$–$C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or C_1–C_6 alkyl;

R^{11} is hydrogen, C_1–C_6 alkyl, phenyl or benzyl;

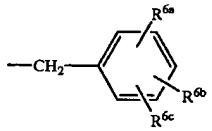
R^{14} is hydrogen or C_1–C_6 alkyl;

R^{50} is hydrogen or (=O);

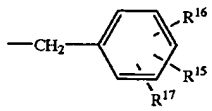
Y is CH, O, NH, S, SO, SO_2 or $(C_1$–$C_3)$alkylamino;

R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

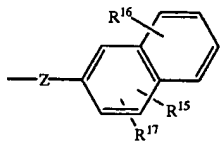
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, halo or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1$–$C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{19}R^{19}$, NO_2, OPO_4H_2, $(C_1$–C_6 alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or C_1–C_6 alkyl;

R^{23} is hydrogen or $(C_1$–$C_3)$alkyl;

Z is —$(CH_2)_n$— or $(C_3$–$C_5)$cycloalkyl;

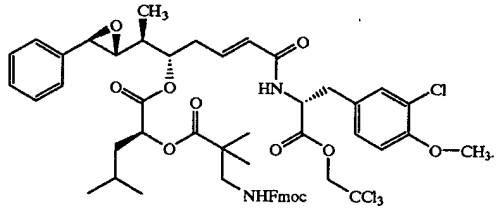
n is 0, 1, or 2;

Z' is an aromatic or substituted aromatic group; and

R^{82} is a base labile protecting group; or a pharmaceutically acceptable salt thereof.

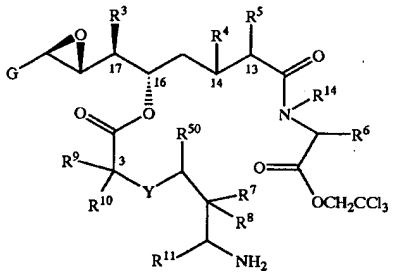
31. A compound according to claim **30** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with —$CH_2OC(O)(CH_2)_mNH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is C_1–C_6 alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA).

32. A compound according to claim **30** wherein said compound is represented by the formula



33. A compound of the formula

(19)



wherein

G is C_1–C_{12} alkyl, C_2–C_{12} alkenyl, C_2–C_{12} alkynyl, or Ar;

Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

R^3 is C_1–C_6 alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^7 and R^8 are each independently hydrogen or C_1–C_6 alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, C_1–C_6 alkyl, C_2–C_6 alkenyl, C_2–C_6 alkynyl, —$(CH_2)_m$—$(C_3$–$C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or C_1–C_6 alkyl;

R^{11} is hydrogen, C_1–C_6 alkyl, phenyl or benzyl;

R^{14} is hydrogen or C_1–C_6 alkyl;

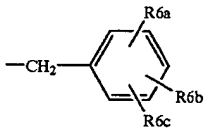
R^{50} is hydrogen or (=O);

Y is CH, O, NH, S, SO, SO_2 or $(C_1$–$C_3)$alkylamino;

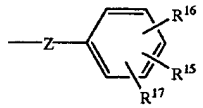
R^6 is C_1–C_6 alkyl, substituted $(C_1$–$C_6)$alkyl, $(C_3$–$C_8)$ cycloalkyl, substituted $(C_3$–$C_8)$cycloalkyl, a heteroaro-

matic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

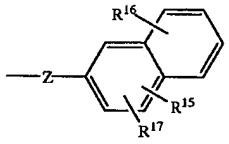
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, halo or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1–C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, $(C_1–C_6$ alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or $C_1–C_6$ alkyl;

R^{23} is hydrogen or $(C_1–C_3)$alkyl;

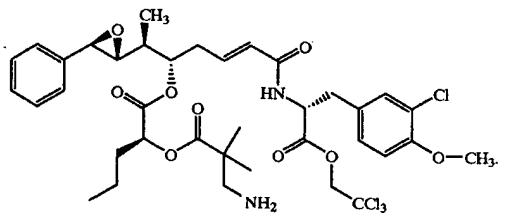
Z is $—(CH_2)_n—$ or $(C_3–C_5)$cycloalkyl;

n is 0, 1, or 2; and

Z' is an aromatic or substituted aromatic group; or a pharmaceutically acceptable salt thereof.

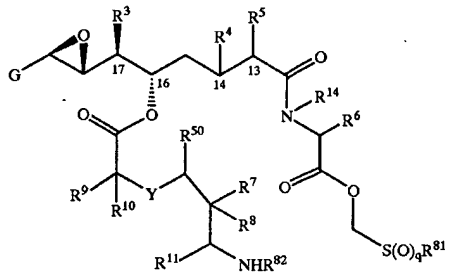
34. A compound according to claim **33** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_mNH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is $C_1–C_6$ alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA).

35. A compound according to claim **33** wherein said compound is represented by the formula



36. A process for preparing a compound of the formula

(9)



wherein

G is $C_1–C_{12}$ alkyl, $C_2–C_{12}$ alkenyl, $C_2–C_{12}$ alkynyl, or Ar;

Ar is an aromatic or heteroaromatic group or a substituted aromatic or heteroaromatic group;

R^3 is $C_1–C_6$ alkyl;

R^4 and R^5 are each hydrogen; or R^4 and R^5 taken together form a second bond between C-13 and C-14;

R^7 and R^8 are each independently hydrogen or $C_1–C_6$ alkyl; or

R^7 and R^8 taken together form a cyclopropyl or cyclobutyl ring;

R^9 is hydrogen, $C_1–C_6$ alkyl, $C_2–C_6$ alkenyl, $C_2–C_6$ alkynyl, $—(CH_2)_m—(C_3–C_5)$cycloalkyl or benzyl, wherein m is the integer one to three;

R^{10} is hydrogen or $C_1–C_6$ alkyl;

R^{11} is hydrogen, $C_1–C_6$ alkyl, phenyl or benzyl;

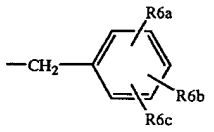
R^{14} is hydrogen or $C_1–C_6$ alkyl;

R^{50} is hydrogen or (=O);

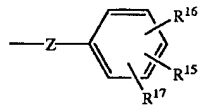
Y is CH, O, NR^{12}, S, SO, SO_2, wherein R^{12} is H or $C_1–C_3$ alkyl;

R^6 is $C_1–C_6$ alkyl, substituted $(C_1–C_6)$alkyl, $(C_3–C_8)$ cycloalkyl, substituted $(C_3–C_8)$cycloalkyl, a heteroaromatic or substituted heteroaromatic group or a group of formula (IA), (IB) or (IC):

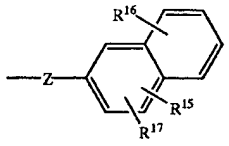
(IA)



(IB)



(IC)



R^{6a}, R^{6b}, and R^{6c} independently are H, $(C_1–C_6)$alkyl, halo $NR^{18}R^{19}$ or OR^{18};

R^{15}, R^{16}, and R^{17} independently are hydrogen, halo, $(C_1–C_6)$alkyl, OR^{18}, O-aryl, NH_2, $NR^{18}R^{19}$, NO_2, OPO_4H_2, ($C_1–C_6$ alkoxy)phenyl, S-benzyl, $CONH_2$, CO_2H, PO_3H_2, SO_2R^{23}, or Z';

R^{18} and R^{19} independently are hydrogen or $C_1–C_6$ alkyl;

R^{23} is hydrogen or $(C_1–C_3)$alkyl;

R^{81} is $C_1–C_6$ alkyl, $C_3–C_8$ cycloalkyl, phenyl or benzyl;

R^{82} is a base labile protecting group;

Z is $—(CH_2)_n—$ or $(C_3–C_5)$cycloalkyl;

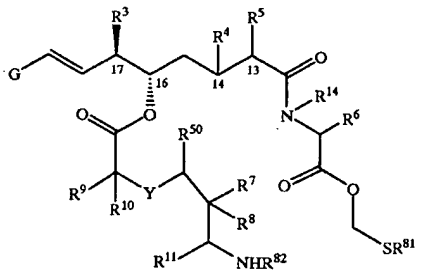
n is 0, 1, or 2;

q is an integer 1 or 2; and

Z' is an aromatic or substituted aromatic group; or a pharmaceutically acceptable salt thereof;

comprising oxidizing a compound of the formula

(11)



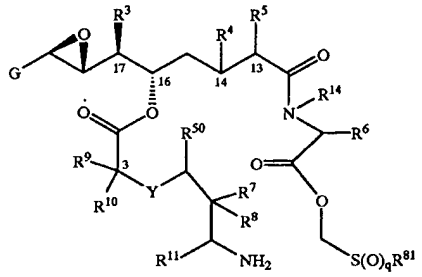
wherein G, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, R^{81}, R^{82} and Y are as defined above, with an oxidizing agent to form a compound of formula (9) and optionally forming a pharmaceutically acceptable salt of a compound of formula (9).

37. A process according to claim **36** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_m.NH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is $C_1–C_6$ alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA) and the oxidizing agent is Oxone.

38. A process for preparing a compound of formula (I) according to claim **36**, further comprising the steps of:

(a) deprotecting a compound of formula (9) with a suitable deprotecting agent to form a compound of the formula

(10)



wherein G, R^3, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{14}, R^{50}, Y, q and R^{81} are as defined above; and optionally contacting a compound of formula (10) with a cyclizing agent to form a compound of formula (I); and

(b) optionally forming a pharmaceutically acceptable salt of a compound of formula (I).

39. A process according to claim **38** wherein G is phenyl, para-fluorophenyl, or phenyl substituted with $—CH_2OC(O)(CH_2)_m.NH_2$; R^3 is methyl; R^4 and R^5 taken together form a second bond between C-13 and C-14; R^9 is $C_1–C_6$ alkyl; R^{10} is hydrogen; R^{11} is hydrogen; R^{14} is hydrogen; R^{50} is (=O); Y is O; and R^6 is a group of the formula (IA); said deprotecting agent is piperidine; said oxidizing agent is Oxone; said oxidant is Oxone and said chiral ketone is a compound of formula (7).

40. A process according to claim **38** wherein said compound of formula (I) is Cryptophycin 52.

* * * * *